Monitoring A Potentially Stressful Situation In Captive Western Lowland Gorillas (gorilla Gorilla Gorilla) Through Analysis Of Behavior and Urinary Cortisol

2006

Douglas Skurski
University of Central Florida

MONITORING A POTENTIALLY STRESSFUL SITUATION IN CAPTIVE WESTERN LOWLAND GORILLAS (*GORILLA GORILLA GORILLA*) THROUGH ANALYSIS OF BEHAVIOR AND URINARY CORTISOL

by

DOUGLAS ANTHONY SKURSKI
B.S. Washington State University, 1998

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
in the Department of Biology
in the College of Arts and Sciences
at the University of Central Florida
Orlando, Florida

Spring Term
2006

ABSTRACT

The concept of quantifying animal welfare has received much discussion, in various industries such as agriculture, laboratory, and zoological facilities. Behavioral, physical, and physiological indicators of welfare have previously been used to assess animal welfare; each having advantages and disadvantages, ranging from the practicality of data collection, to the validity of the data and how it is interpreted. Concurrent assessment of multiple measures is a more robust way to examine animal welfare, which utilizes the advantages of each measure, and provides additional information on which to base conclusions and animal care management decisions.

This study used measures of behavior and urinary cortisol to examine the potential stress response of a captive gorilla group to short-term space restriction associated with temporary confinement to indoor housing facilities. The study duration was three months; one month of baseline data collection, one month of indoor restriction, and one month of monitoring post-restriction. All-occurrences of selected behaviors were collected, with an emphasis on social and stress-related behaviors, and urine samples were collected daily from a sub-set of the group. A urinary cortisol metabolite enzyme immunoassay was validated and used to monitor adrenal activity in gorillas. Measured cortisol increases in response to a known stressor (medical illness) provided a physical validation of the cortisol EIA and established biological relevance of the assay system.

No significant differences in social behaviors (aggression, affiliation) or stereotypic behaviors were observed. Significant ($p<0.05$) increases in cortisol

concentration were measured, suggesting that the gorillas were responding to a stressor during the study period. The observed cortisol increase was not likely to have been caused exclusively by the temporary indoor confinement. Potential additional causes of increased adrenal activity during the study included: presence of the observer and novelty of re-landscaped outdoor enclosure.

While the increases in cortisol concentration demonstrate an observed stress response, the magnitude of this stressor, and thus the degree of the stress response, was minor. The stress experienced was not significant enough to alter the normal biological function of the gorillas, and thus, can be considered negligible. The gorillas' ability to effectively deal with this expected stressor may have been enhanced by the additional enrichment provided to the gorillas during their indoor confinement. Gorillas were provided with additional browse, more enrichment items, additional training sessions, and increased keeper interaction while they remained indoors. These animal care and management techniques may have buffered the predicted negative impact on animal welfare due to increases in stress by providing stimulating novelty in the gorillas' indoor environment.

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

.

LIST OF ABBREVIATIONS

ABTS – 2,2"-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

ACTH – adrenocorticotrophic hormone

AVP – arginine vasopressin

BE – Benga

BSA – bovine serum albumin

CHRP – cortisol-horseradish peroxidase

CRH – corticotrophin-releasing hormone

DAK – Disney's Animal Kingdom

EIA – enzyme immunoassay

GI – Gino

HA – Hasani

HO – Hope

HPA – hypothalamic-pituitary-adrenal

JA – Jabari

MK – Makena

OB - Observer

RIA – radio immunoassay

WTC – Wildlife Tracking Center

LITERATURE REVIEW

This research focused on the monitoring of a captive gorilla group through a potentially stressful temporary space restriction. Literature review of essential background topics; gorilla natural history, stress, animal welfare, and space restriction, was conducted and summarized.

<u>Gorilla Natural History</u>

Gorillas have traditionally been classified as three sub-species; western lowland gorilla (*Gorilla gorilla gorilla*), eastern lowland gorilla (*Gorilla gorilla graueri*), and mountain gorilla (*Gorilla gorilla beringei*). Recent taxonomic reclassification groups gorillas into two species and four sub-species: western gorillas: western lowland gorillas (*Gorilla gorilla gorilla*) and Cross River gorillas (*Gorilla gorilla diehli*); eastern gorillas: eastern lowland gorilla or Grauer's gorilla (*Gorilla beringei graueri*) and mountain gorilla (*Gorilla beringei beringei*) (Groves, 2001). Western lowland gorilla is the sub-species represented in most captive gorilla populations, as very few Grauer's gorilla and no mountain gorilla or Cross River gorillas are known to exist in zoological facilities.

Until recently, most of what was known about gorillas was learned through study of mountain gorillas. Recently, scientists have reported more success in the *in situ* study of western gorillas. Discussion of western gorilla natural history is still largely extrapolated from knowledge of eastern gorillas, though known differences are clarified where possible through recent research. Wild western lowland gorillas exist in the tropical forests, seasonally inundated forests, and even swamp forests of central west Africa, inhabiting average home ranges of approximately 25 km^2 (Tutin, 1996). Their

1

diet is primarily frugivorous, supplemented with foliage during seasons in which fruits are less plentiful (Doran, et al 2002; Remis, 1997). Western gorillas primarily occur in one of three social contexts: family groups, all-male groups, or solitary males (Gatti, et al. 2004; Parnell, 2002; Robbins, et al. 2004). The primary social group consists of one adult, dominant "silverback" male, an average of three adult females, and their offspring (Harcourt, 1981; Parnell, 2002). Groups may also consist of multiple, often related, adult males, such as brothers or father/son pairs (Robbins, 1999). On average, most groups contain 5-10 individuals, though groups of 29 or more have been reported (Gatti, et al 2004; Magliocca, et al 1999, Parnell, 2002; Robbins, et al 2004). These are stable, cohesive groups with minimal male-female or female-female agonistic interaction within group, though agonistic encounters do occur between groups. Affiliative interactions between females within a group are rare. Observed affiliation occurs more often between females and the breeding male, or with and among offspring (Stokes, 2004).

Most male offspring remain with their natal troop until roughly 6-8 years of age at which time they emigrate from their natal group to all-male groups or range as a solitary male. Male offspring may also remain in their natal group, and "sneak" copulations with unrelated females within the group. Female offspring emigrate, also around 6-8 years of age, from their natal group directly to another adult male to form a breeding group, or transfer into an existing group (Stokes and Parnell, 2002).

Stress

The word "stress" has been used as a generic term to describe many types of negative or positive stimulation as well as an individual's behavioral, physiological, or psychological response to that stimulation. Because of the broad range of uses, the term

"stress" has a multitude of definitions, depending upon the context (Broom and Johnson, 1993; Moberg, 1987). Hereafter, stress will be defined as the biological process by which an individual attempts to cope with a real or perceived threat to physiological or psychological integrity (Broom and Johnson, 1993; McEwen, 2000), and the real or perceived threatening situation that elicits this biological process, or stress response, will be referred to as a "stressor."

Despite the negative connotations commonly associated with the term "stress," not all stress is detrimental. Stress is an evolved adaptation: an individual's biological defense to cope with stressors. Moberg (1999) presented a model of stress that illustrates the process and divided stress into three general stages: the recognition of a stressor, the biological defense against the stressor, and the consequences of the stress response. During the first stage, the recognition of a stressor, the central nervous system perceives a stressor. The second stage is the biological response to the stressor, which may include a behavioral, autonomic, neuroendocrine, or immunological response. This response changes the normal biological function (pre-stressor) to an altered biological function (post-stressor) at a certain biological cost. If the stressor is minor, or of short duration, the individual's biological reserves may be adequate to absorb the cost of the stress without disrupting normal biological function. If the stress response is effective in alleviating the stressor, biological function will quickly return to normal. For instance, if a prey species, such as a gazelle, recognizes a predator, a predatory stress response ensues. The appropriate biological response is likely be behavioral - to run away. If that stress resulted in the appropriate behavioral actions required to avoid predation, the stress was beneficial, because the cost of the stress response – running – was insignificant

compared to the benefit – survival. This type of stress, in response to a stressor of short duration, is described as acute stress.

However, not all stressors can be dealt with as efficiently. During prolonged or severe stress, otherwise referred to as chronic stress, the biological reserves may be insufficient to absorb the cost of the stress. Biological resources must then be diverted from the pre-stress activities of normal biological function, such as growth, immune function, or reproduction, and concentrated on the stress response. In Moberg's (1999) model of stress, this altered biological function makes up the third stage; the consequences of stress. When dealing with chronic stress, the biological cost is significant. In diverting energy from normal biological functions to accommodate the continuing stress, those normal functions may be suppressed. Moberg describes this disruption as the "pre-pathological state." In this state, the individual is at risk for developing pathologies, such as stunted growth, disease, suppressed reproduction, or stereotypic behavior. When the stress response enters the pre-pathological or pathological states, it ceases to be adaptive and beneficial, and the animal is said to be in the state of "distress." Distress will last until the stressor is alleviated and the animal restores biological reserves sufficiently to resume normal biological function.

The primary neuroendocrine system behind the stress response is the hypothalamic-pituitary-adrenal (HPA) axis. When a stressor is sensed by the brain, it triggers the hypothalamus to release two hormones, corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). These hormones cause the anterior pituitary gland to release adrenocorticotrophic hormone (ACTH), which in turn, stimulates the adrenal cortex to secrete corticosteroids (cortisol/corticosterone) (Buckingham, 2000).

During a stress response, these corticosteroids divert energy from normal biological functions and concentrate energy on coping with the stress. Cortisol is one of the corticosteroids that have been recognized as useful in indicating stress (Carlstead, et al. 1992; Thomas and McCann, 1997; Wielebnowski et al., 2002). Patterns of cortisol excretion have been successfully measured in blood (Boinski, et al. 1999), urine (Czekala, et al. 1994), feces (Wasser, et al. 2000), and saliva (Kuhar, et al. 2005) from various animal species.

Animals in captivity may experience situations that they perceive to be stressful, despite the best efforts of zoos and aquariums. Some events, such as the transfer of animals from one institution to another, veterinary procedures, or social group changes are unavoidable in order to effectively care for the individual animal or the species' captive population. Occasional acute stress is not necessarily harmful. Some forms of acute stress have been shown to be beneficial in facilitating reproductive activation, enhancing alertness and exploration, and improving immune response (Moodie and Chamove, 1990; Weiss, et al 1989). Conversely, repeated acute stress or prolonged, chronic stress has been shown to suppress reproduction, reduce immune effectiveness, and cause the development of aberrant behaviors (Moberg, 1985).

Animal Welfare

The concept of animal welfare is one of political and ethical importance. Despite the many attempts to address welfare, the issue remains ambiguous (Mason and Mendel 1993). This confusion stems from the philosophical disagreements of how to define animal welfare, and the scientific problems inherent in trying to objectively measure it.

Definitions and metrics tend to focus around two central themes, physical and psychological health.

The inability to agree on a definition of animal welfare is problematic. Some scientists have equated welfare with biological fitness, stating that an animal has poor welfare only if the animal's ability to survive and reproduce is compromised (Barnett and Hemsworth, 1990). By this definition, factors that would diminish welfare include damage to the body, such as cuts, bruises, and broken bones, or disease. Broom (1991) agrees with defining welfare by physical health, but expands the definition by adding that an animal's fitness can be reduced if it is unable to cope with its environment. This acknowledges that mental states such as fear, anxiousness, frustration, or boredom can have detrimental consequences to physical health. Other scientists believe that the mental state of the animal is the primary concern and concentrate on psychological health in their definitions (Dawkins 1988; Duncan and Petherick 1989; Sandoe and Simonsen 1992). A poor mental state can result in physiological responses to stress, stereotypic behaviors, and lethargy. In summarizing the available research on animal welfare, the American Zoo and Aquarium Association (AZA) Animal Welfare Committee developed the following "working definition" of animal welfare (Barber and Mellen, pers. comm.):

> "The degree to which an animal can cope with challenges in its environment as determined by a combination of veterinary measures of health (including pre-clinical physiological responses) and measures of psychological well-being."

The definition a scientist prefers has a direct influence on the measures employed to objectively assess welfare. Criteria used to assess physical health are relatively simple. Wounds are visible and can be noticed during routine inspections. Disease can be assessed by performing blood or other types of test (e.g., veterinary pathology). Also, an

animal usually behaves differently when ill, and these behavioral changes can be observed. For example, a cow with abdominal pain often lies down and stands up repeatedly, takes on an unusual posture, or stands pushing its heads against a wall (Hart 1987). It is much more difficult to objectively measure the subjective feelings of animals, as is necessary to address the psychological health definition of welfare. Attempts have been made to link behavior and physiological responses to situations that the animal must find unpleasant. In the presence of a stressor, such as food deprivation, electric shock, dehydration, sensory deprivation, etc., changes in behavior and physiology can be measured. If the animal exhibits similar responses in another situation, it is inferred that the animal finds that situation correspondingly "unpleasant" (Mason and Mendel 1993). Additionally, an animal's wants or desires can be related to its motivation. Motivation can be measured by preference experiments and consumer demand tests. Allowing the animal to choose between objects, resources, or locations and recording the amount of work the animal is willing to do to achieve that choice gives insight towards the motivation of that animal (Dawkins, 1990).

Fraser (1995), realizing these differing viewpoints, pondered whether measures of animal welfare from different viewpoints could be unanimously accepted. He gave an example of an unrelated, complicated problem to be addressed and looked at it through three thought process "concepts." A Type 1 concept was one that consisted of a single attribute, and could be measured directly, such as height, or mass. A Type 2 concept was a single attribute that could not be measured directly, but could be approximated by combining measurements of contributing variables. An example of a Type 2 concept could be the mass of the moon. This single attribute cannot be directly measured using

7

any existing resource, but can be accurately approximated based on other measurable factors. A Type 3 concept involves "multiple attributes linked by some commonality in function." Such a concept is not directly measurable, although attempts to quantify can be made by combining a number of attributes to create some overall index for that concept. By this analogy, animal welfare is a Type 3 concept. It is not a single attribute, and is not directly measurable by a single criterion, but by measuring multiple attributes that make up animal welfare, and combining those measures together, we can make the best possible estimate of an animal welfare "index" measure.

An animal with optimal welfare should have the opportunity to express naturally occurring, species-appropriate behaviors in response to the relevant conditions or stimuli. To monitor the presence or absence of species-appropriate behaviors, an ethogram (an exhaustive catalogue of behaviors that the species is capable of performing) must be compiled (Banks, 1982). Methodical observation can then used to compile a time budget to compare the behavior of the animal to other individuals using the same ethogram to see if there is any deviation. Deviation could be the absence of certain expected behaviors, or the addition of non-appropriate, behaviors such as stereotypy.

Physiological data is typically used to assess stress levels. A stress response begins in an animal when the central nervous system perceives a threat to homeostasis. The autonomic response affects a number of biological systems including the cardiovascular system, the gastrointestinal system, and the exocrine glands (Moberg, 2000). Measurable responses to this system include changes in blood pressure, heart rate, and body temperature (Schnell and Wood, 1993). These responses to stress are of a relatively short duration, and may not have a large impact on the overall welfare of an

animal. However, the hormones secreted by the hypothalamic-pituitary neuroendocrine system have a broad, long-lasting effect on the body. The hypothalamic-pituitary-adrenal (HPA) axis has been the primary neuroendocrine axis monitored. Secretion of cortisol and corticosterone from this axis has been observed in response to stress (Clark, et al 1997; Thomas and McCann 1997; and Wasser, et al 2000). These hormones can be measured in blood, saliva, urine, or feces (Moberg, 2000). Obtaining samples from many of the available bodily fluids or excretions may involve handling, probing, injecting, or other procedures invasive to the animal, which has been found to confound the data (Cook, et al 2000). Hormones, however, secreted in urine and feces can be collected non-invasively, preventing disturbance to the animal. The hormones can then be extracted from the waste product to give useful information about the stress levels of an animal (Wasser, et al 2000).

Space Restriction

Environmental changes, such as a reduction in the amount of available space, are additional examples of potential stressors that have been examined. Traditionally, researchers used rats as test subjects to examine the effects of space restriction and crowding (Calhoun 1962). This initial research placed an expanding rat population in a limited space enclosure and observed a drastic increase in aggression as density increased, eventually resulting in rats attacking, killing, and finally cannibalizing each other. Calhoun (1962) concluded that as population densities increase, so to do aggressive interactions. Views of density-induced aggression were soon generally extrapolated across all animals, including humans (de Waal, 1989).

However, studies on non-human primates do not support a simple relationship between population density and aggression levels (de Waal, 1989; de Waal, et al. 2000). Many social primates obtain benefits from, and thus place value in maintaining, social relationships. Aureli (1991) reviewed several hypothesized primate social mechanisms, in both captive and wild animals, for coping with social tension due to agonistic conflicts, and restoration of social homeostasis through reconciliation as a means to maintaining important social bonds. Similarly, primates tend to employ one of two coping strategies to reduce aggression under space restrictions and increased densities. In captivity, under short-term confinement, chimpanzees (*Pan troglodytes*) (Aureli and de Waal, 1997), macaques (*Macaca fascicularis*) (Aureli et al, 1995), and squirrel monkeys (*Saimiri sciureus*) (Perloe, 1986) have displayed a conflict avoidance strategy, aimed at reducing the risk of aggression by decreasing the overall level of movement and social activity in an environment in which forced proximity and limited escape opportunities increase the opportunity for conflict. Use of this strategy by primates in these studies resulted in an observed reduction in affiliative behaviors and an increase in submissive behaviors. Under long-term space restriction (a full season or longer), rhesus monkeys (*Macaca mulatta*) (Judge and de Waal, 1997) and bonobos (*Pan paniscus*) (Sannen, et al. 2004) were observed to utilize a more active tension-reduction strategy, such as increased affiliative allogrooming. Increased allogrooming functions to relieve tension caused by higher densities, and thus lower the potential for aggression.

While these studies reported an active strategy to reduce aggression during space restriction, they do not imply that these primates are experiencing optimal welfare. To the contrary, maintenance of social bonds in this manner comes at a cost. These primates

are restricting their normal behavioral repertoire in an effort to minimize aggressive encounters, and in the process, are experiencing increased stress. Stress in chimpanzees under higher densities was measured by rates of a stereotypic behavior (self-scratching) and physiological indicators (fecal cortisol) during short-term space restriction. Increased stereotypic behavior and cortisol levels during high densities due to space restriction indicated that the strategies used to reduce agonistic encounters resulted in a stress response in those individuals (de Waal, et al. 2000).

INTRODUCTION

It is a primary responsibility of modern zoos and aquariums to provide optimal care for the animals in their collections (Maple, et al. 1995). Providing optimal care includes maintaining an animal's physical health, through proper nutrition, adequate enclosures, and quality veterinary attention. However, optimal care is not achieved through physical health alone. Zoos and aquariums must also maintain the psychological health of their animals by providing species-appropriate social opportunities, naturalistic enclosures, and effective enrichment and training (Mench and Kreger, 1996; Mellen and Sevenich-MacPhee, 2001). Within the zoo environment, the intention is to optimize physical and psychological health to enhance animal welfare.

As the zoo community has become growingly more conscious of the multi-faceted concept of animal welfare, many attempts have been made to define or describe what constitutes animal welfare, both good and bad. Zoo research professionals, in pursuit of ways to measure animal welfare, contribute to a growing body of empirical research termed "zoo animal welfare research" (Shepherdson et al. 2004). Researchers use a variety of physical, behavioral, and physiological measures, which, when analyzed in combination, and interpreted in the context of what is appropriate for the subject species, provide indications as to the status of an animal's well-being.

Measures often used to examine animal welfare include behavioral monitoring (Akers and Schildkraut, 1985; Lutz, et al 2003; Nash, et al 1999; Woods, 2001) and hormonal analysis of the hypo-pituitary-adrenal (HPA) axis; which is responsible for production of glucocorticoids in response to a stressor (Barnett and Hemsworth, 1990;

Broom and Johnson, 1993; Thomas and McCann, 1997). It can be difficult to interpret animal welfare implications from measures of behavior or physiology alone. Behavioral observations can be unreliable indicators of stress, due to individual variation in stress reactivity, variation in coping strategies, and researchers' basic lack of understanding of the causal mechanisms underlying observed behavioral changes (Rushen, 2000). Interpretation of glucocorticoid levels as indicators of stress can be similarly unreliable. Both positive (e.g., copulation) and negative (e.g., injury or illness) experiences result in a temporary elevation in glucocorticoids (Wielebnowski, 2003). Further, determination of the amount of change to glucocorticoid levels required to cause a harmful effect to an animal's welfare is variable dependent upon the stressor, species, and individual (Rushin, 1991). Finally, measured decline in glucocorticoid levels, may be the result of negative feedback hormonal control mechanisms, rather than the elimination or reduction in the external stressor (Smith, 2002). Despite the inherent drawbacks of each measure independently, the assessment of multiple measures of animal welfare can provide additional insight into the impact of various stressors on animal well-being, and lead to a more informed animal welfare conclusions (Pedersen, 1996; Shepherdson, et al 2004; Wielebnowski, 2003).

Zoo professionals have applied these various techniques to examine aspects of the welfare of the animals in their care. A few examples of issues examined include: environmental enrichment, factors influencing reproductive success, effects of social dynamics/group composition, expression of stereotypic behaviors, etc.

Environmental enrichment encompasses a broad range of activities intended to provide for the physical, psychological, and behavioral needs of captive animals. The

goal of an effective environmental enrichment program is to allow for the expression of species-appropriate behaviors, reduce or eliminate aberrant behaviors, identify and reduce potential chronic stress and enhance an animal's ability to successfully cope with acute stressors, and by accomplishing these goals, improve an animal's overall welfare (Carlstead and Shepherdson, 2000; Mellen and MacPhee, 2001). Studying brown capuchins (*Cebus paella*), Boinski, et al. (1999) quantified the effectiveness of environmental enrichment at reducing stereotypic behaviors and reducing stress, through measures of behavior and plasma and fecal cortisol. They found that stereotypic behavior and cortisol levels decreased as enrichment increased in amount and complexity.

Animal welfare, manifested in effects on reproduction, has been studied in felids. In a multi-institution study of 20 small felid species, behavioral observations were used to conclude that insufficient environmental conditions, such as inappropriate group size or frequent illness, negatively correlated with reproductive success (Mellen, 1991). Wielebnowski et al. (2002) examined multiple chronic stressors and the behavioral and physiological consequences in clouded leopards (*Neofelis nebulosa*). Similar studies have been conducted in other species, including cheetahs (*Acinonyx jubatus*) (Wielebnowski, 1999), black rhinoceros (*Diceros bicornis*) (Carlstead, et al. 1999), and honeycreepers (*Vestiaria coccinea*) (Shepherdson, et al 2004).

The effects of stressful social situations on animal welfare have also been examined in many species, but most commonly in primates and elephants. Dominance hierarchies, and the effects to subordinate individuals, have been examined in orangutan (*Pongo pygmaeus*) (Maggioncaida, et al. 2002), rhesus macaques (*Macaca mulatta*) (Bercovitch and Clarke, 1995), and primates in general (Abbott, et al. 2003). Changes to

group social dynamics, such as removal of individuals from a group, addition of individuals to a group, high population density due to space restriction, or social isolation of an individual has been shown to be stressful events, resulting in increased aggression (Burks, et al 2001; Hoff, et al 1996), decreased affiliative behaviors (Aureli et al, 1995; Aureli and de Waal, 1997; Perloe, 1986), increased stereotypic behaviors (Bowen, 1980; Schmid, et al. 2001), and increased levels of cortisol (Kuhar, et al. 2005; Stoinski, et al. 2002; Ziegler, et al. 1995). In western lowland gorillas, Kuhar, et al. (2005) monitored behavioral and cortisol changes in an all-male group, and Stoinski, et al. (2002) examined differences in cortisol levels in males of different ages and males living in different social settings (i.e., solitary males, all-male groups, or heterosexual groups). Both studies measured the stress of various social settings on male gorillas through the physiological measure of cortisol.

The present study examined stress and aggression before, during, and after temporary spatial restriction in western lowland gorillas (*Gorilla gorilla gorilla*). For exhibit maintenance purposes, the gorillas were prevented from accessing their outdoor facilities and restricted to their indoor holding enclosure for one month. This change in the group's daily routine and restriction to more confined space indoors was a potential stressor to the gorillas. The primary objective of this study was to quantify the potential stress due to short-term spatial restriction using concurrent measures of behavior and urinary cortisol. This study also provided behavioral data that allowed comparisons of strategies to cope with crowding used by gorillas, to crowding coping strategies used by primates of different social structures. It was predicted that temporarily restricting the gorilla group to their indoor housing would result in increased aggression and decreased

15

affiliative behaviors between gorillas, increased stereotypic and solitary behaviors, and increased urinary cortisol concentrations. Upon return to their outdoor habitat, aggression, affiliative behavior, stereotypic behavior, and urinary cortisol concentrations were predicted to return to baseline levels.

METHODOLOGY

The subject of this study was a 3.3 (male.female) group of western lowland gorillas (*Gorilla gorilla gorilla*) at Disney's Animal Kingdom (DAK); Lake Buena Vista, Florida. Table 1 provides summarized group information. The outdoor habitat of this gorilla group was re-landscaped with small trees, shrubs, and sod. The time allotted to perform these modifications, and to allow time for the vegetation to establish, was one month. During this replanting of their outdoor habitat, a gorilla group was confined to their indoor holding area. This research monitored the members of this gorilla group through this period of confinement indoors, with the purpose of determining if the prolonged confinement to their indoor enclosure was stressful to the gorilla group.

Experimental Design

To empirically address this question, behavioral and physiological data were collected over a period of three months; one month prior to, one month during, and one month after the indoor confinement. The one-month period prior to confinement was to establish the behavioral and physiological baselines. These baselines measures were intended to establish the behavioral patterns and cortisol levels of each individual during routine conditions. The one-month period during confinement was the experimental treatment. Any changes in behavior or cortisol as a result of this modification to the gorilla group's daily routine were to be determined by comparing data from this treatment condition to the baseline condition. To verify that the baseline data were representative of the individuals during routine conditions, data were collected for the one-month period following confinement, when the group's daily housing schedule

17

returned to normal. During this period, a predicted return of behavior and cortisol to baseline levels was predicted, after the novelty of their modified outdoor exhibit diminished.

Animal Housing

The gorilla group was exhibited in a 0.30-hectare (0.75 acre) naturalistic enclosure. The outdoor habitat was connected through a shift door inside a rock cave to a 0.008-hectare (855 sq ft) indoor holding area, comprised of four interconnected rooms. At daily park closing, the group was shifted indoors, through the cave shift door.

Behavioral Data Collection

Sampling Method

A species-specific ethogram was used to identify and define the behaviors to be recorded in this study (Appendix A). Continuous, or all-occurrence, sampling method (Altmann, 1974; Martin and Bateson, 1993) was used in this study. All occurrences of behaviors listed on the ethogram were recorded for all six gorillas during each observation period. Both initiator and receiver were identified during behavioral interactions. For behaviors that were defined as states, rather than events, occurrence of those behaviors were counted as one bout regardless of the duration. If there was a gap of five or more seconds between bouts, then those bouts were scored as separate occurrences of the state-like behavior. This allowed for the data to be analyzed for frequency and rates of behavior. Each observation period was one hour in duration. The one-hour observations were divided into four 15-minute quarters. If any animal was not visible for the duration of any quarter, Not Visible (NV) was scored for that quarter. If

18

an animal was visible for all or part of any quarter, regardless of whether or not behaviors from the ethogram were exhibited, the animal was considered visible for the quarter.

Data were recorded simultaneously using two techniques: direct observation and video recording. In both the outdoor habitat and the indoor holding area, it was not possible for a single observer to see all enclosures at the same time. In order to record all occurrences of specified behaviors for all animals in the group, video cameras were utilized to record some areas of the enclosure concurrently with direct observations. Data from the video tapes for each hour of observation were scored to supplement data collected during direct observation, to determine the total number of behaviors recorded in that hour. These data were used to calculate rates of behaviors per hour.

Data Collection Schedule

During the three-month study, data were collected during three one-hour observation periods between the hours of 06:45 and 12:00, five days per week. A minimum of 60 hours of data per month was collected for the three-month duration of the study. A schedule for data collection is included in Table 3. As shown in Table 2, Disney's Animal Kingdom opened one hour earlier for a period of 13 days from March 25 to April 6, 2002 to accommodate a large increase in guest attendance associated with Spring Break. As a result, the gorillas were released into their outdoor enclosure one hour earlier during those days and the data collection also began one hour earlier.

Urine Collection and Processing

Urine samples, from a sub-set of the study group, were collected during the experiment and were analyzed for the hormone cortisol. Due to the circadian nature of cortisol secretion in gorillas, it was important that all samples were collected at the same

time each day. Cortisol concentrations in gorilla urine are highest in the early morning hours and lower as the afternoon and evening progress (Czekala, et al 1994, Muller and Lipson, 2003; van Eekelen, et al 2003). Keepers used operant conditioning techniques to train the gorillas to urinate on cue. Each morning, between 06:30-07:30, two keepers attempted to collect urine from the gorillas. The urine collected at this time was presumably the first void of the day, thus having the highest concentration of cortisol. Collection protocol included keepers asking each gorilla to position itself at an individual station at the front of the enclosure. Each animal was given a food reward when it urinated. As the urine ran out of the enclosure, the keepers collected it by aspirating the urine from the floor with a plastic syringe. Volume of an average urine sample was approximately 2 mL. The sample was transferred to a Sarstedt tube, labeled with the animal's name, ID number, date of sample, and sample number, and stored in a freezer at -20°C to preserve the hormone samples until they could be analyzed at a later date.

The collection efforts focused on the four oldest animals (BE, GI, HO, and HA), each already trained to perform the urination behavior. The two youngest gorillas (JA and MK) had not been trained to perform this behavior at the time of the study, and no training attempts were to be made during the course of this study. Therefore, urine samples were not collected from the two youngest animals. Keepers attempted urine collection from each of the four oldest gorillas every day through the three month duration of the study.

Cortisol Enzyme Immunoassay

Cortisol enzyme immunoassay (EIA) for gorillas was developed and assays performed at Disney's Animal Kingdom Wildlife Tracking Center (WTC). While

cortisol has been previously examined in gorilla urine (Bahr, et al 1998; Czekala, et al 1994; Robbins and Czekala, 1997; Stoinski, et al 2002), these studies all utilized radio immunoassay (RIA). The competitive cortisol EIA employed in this study to measure cortisol concentration was a modification of the assay developed by Munro and Stabenfeldt (1985). When establishing a new assay protocol, it is important to validate the test for the species being examined and the medium containing the hormone (Buchanan and Goldsmith, 2004). This modified assay protocol was validated for gorilla urine, to assure that the antibody in the assay has a broad enough cross-reactivity to bind to the modified form of cortisol excreted in gorilla urine. The assay was validated by establishing parallelism between the slope of the standard curve and slope of the serial dilution of unknown samples. This parallelism indicated that the metabolites extracted from the urine were interacting with the antibody similarly to the cortisol standards used in the EIA.

In an additional step to validate the assay protocol, the physiological relevance of the measurements was to established, demonstrating the cortisol metabolites were reflecting adrenal function. Typically, this would have involved injecting ACTH into the subject, to "challenge" the HPA-axis and stimulate the production of cortisol. If the assay was then able to report elevated cortisol levels, as expected after the challenge, the physiological relevance of the measurements would be confirmed. Due to the invasiveness of the challenge, this procedure was not an acceptable option for this study at DAK. At the study's outset, physiological relevance was not directly observed in this assay, but instead, was inferred from that of previous studies which used RIA to analyze urinary cortisol in gorillas (Bahr, et al. 1998; Czekala, et al. 1994; Robbins and Czekala,

1997; Stoinski, et al. 2002). However, physiological relevance was demonstrated in this assay opportunistically, at the end of the study. After the study concluded, urine samples continued to be collected and analyzed, for additional comparison at a later date. Approximately two weeks after the study concluded, the "silverback" gorilla (GI) became sick with shigella, a bacterial infection leading to severe diarrhea, dehydration, and fever. Measures of cortisol from samples corresponding to this illness were extremely elevated (3x higher), as would be expected in response to an illness. This confirmed that the gorilla urinary cortisol EIA developed and used in this study was capable of monitoring gorilla adrenal function. Cortisol data corresponding with the period of illness will be presented in the Results section of this paper.

With the EIA validated for gorilla urine, assays on samples were conducted. Micro titer plates were prepared for use in the assay at least one day prior to use. Plates were coated with cortisol antibody (R4866, raised in rabbit against cortisol-3-. carboxymethyloxime:BSA developed by Munro and Stabenfeldt, diluted to 1:8500 with bicarbonate buffer, pH 9.6). 50µl of antibody was pipetted into each well (except the blanks), and incubated overnight or longer at 4°C. Each plate was used within two days of coating.

Standards and samples were prepared on the day they are to be assayed. To prepare standards, cortisol (Reference Prep H5885 Sigma-Aldrich, St. Louis, MO) stock solution (0.1mg/mL cortisol) was diluted to 1000pg/50µl. The top standard was then serial diluted to give standards (all concentrations in pg/50µl) of 500, 250, 125, 62.5, 31.25, 15.75, 7.88, and 3.94. To prepare each gorilla urine sample, the urine was diluted to 1:50 with phosphate buffer solution. To use the plate in the assay, the antibody

solution was discarded from the wells, and each well was washed three times by pipetting 250µl of wash solution (normal saline with 0.5% Tween, from Sigma). 50µl of phosphate buffer was pipetted into each of 96 wells. Twenty-four wells without bound antibodies (blanks) were included on each plate to calculate non-specific binding. These wells received an additional 50µl of phosphate buffer. Four cortisol-horseradish peroxidase (CHRP)-only wells (zeros) were included on each plate to measure 100% binding. Like the blanks, the zeros received an additional 50µl of phosphate buffer, but unlike the blanks, the zeros wells had been coated with antibodies. 50µl of two known concentrations of cortisol (10 pg/50µl and 250 pg/50µl) were added to the plate in two wells per concentration. Those four wells served as controls. 50µl of standards or samples were added to the remaining 72 wells. All 96 wells received 50µl of 1:70,000 CHRP solution, for a total well volume in each well of 150µl. The standards and samples were then allowed to incubate overnight.

After incubation, the plate contents were discarded and the plate was washed three times with 250µl wash solution per well. 100µl of substrate solution was then dispensed to all wells. Substrate solution was made immediately prior to use, and consisted of 12.5ml Citrate buffer (9.61g citric acid (anhydrous Sigma C-0759) in 1L H_2O, pH 4.0), 125µl ABTS solution (0.55 g ABTS (Sigma A-1888) in 25ml H_2O, pH 6.0), and 40µl working H_2O_2 solution (0.5ml of 30% H_2O_2 in 7.5ml H_2O). Plates were gently agitated on a plate shaker for 50 minutes. The plates were removed from the plate shaker, and read on a Molecular Devices Emax (model E9996) plate reader at 405 nm (650 nm reference).

Intra-assay coefficient of variation was 9.2% (n=38) and the inter-assay variability was 10.0% (n=19). Reported antibody cross-reactivity was: cortisol (100%), prednisolone (9.9%), prednisone (6.3%), compound S (6.2%), cortisone (5.0%), and corticosterone (0.7%) (Munro and Stabenfeldt, 1985). Cortisol measures were referenced with creatinine (Jaffe reaction; Taussky, 1954) to account for differences in urine concentration in each sample. Details of the creatinine EIA are available in Appendix B.

Statistical Analyses

Distribution of behavioral and physiological data collected was non-normal, as sample sizes were small and unevenly distributed. Thus, non-parametric statistical tests were used to analyze behavioral and physiological data, as well as the correlation between them. XLSTAT 7.5.3 for Windows and SPSS 10.0 for Windows Student Version were used for the statistical analyses.

Behavioral Data

Behavioral data were grouped into four categories: active aggression, passive aggression, affiliative behaviors, and stereotypic/solitary behaviors. All occurrences of each behavior within each category were summed for each hour of observation, resulting in a rate per hour of each behavioral category per animal. Within each behavioral category, the rates were then averaged within each of the three experimental treatment periods.

Behavioral data were analyzed based on the mean rate of behavior per individual animal (n = 6). Within each behavioral category, Friedman's non-parametric ANOVA ($Q_{0.05, crit}$) was used to compare the mean rate of behavior per individual between each of the three experimental conditions (Zar, 1999). If Friedman's test revealed a significant

difference among the three experimental conditions, a Bonferroni post hoc test was conducted to determine between which conditions the significant difference occurred.

Cortisol Data

Cortisol data were analyzed based the high concentration, low concentration, and mean concentration of cortisol for each experimental condition, per individual gorilla (n = 4). Friedman's non-parametric ANOVA ($Q_{0.05, crit}$) were used to compare the data per individual for each of the three experimental conditions. If Friedman's test revealed a significant difference among the three experimental conditions, a Bonferroni post hoc test was conducted to determine between which conditions the significant difference occurred. As no urine samples were collected for the two juveniles, JA and MK, these individuals were not included in this analysis.

Behavioral and Cortisol Correlation

For primates, urinary cortisol represents the production and excretion of cortisol in response to the previous day's events, as primates have been shown to excrete approximately 90% of all cortisol produced in response to an experimental challenge, within 24 hours of a challenge (Bahr, et al. 2000). Therefore, to analyze relationship between cortisol and behavior, for each individual, cortisol concentrations were compared to behavioral rates from the previous day. Spearman's rank correlation (r, $p < 0.05$) was calculated to determine if rates of behavior and cortisol concentrations co-varied. Observed changes in behavior that correlated with hormonal changes during the indoor confinement, or treatment phase of the experiment, as compared to the pre- and post-treatment phases of the experiment were assessed.

RESULTS

<u>Behavioral Data</u>

A total of 184 hours of data was collected on the group for the three-month duration of the study (approximately 60 hours/month). Varying levels of visibility among the individuals in the group resulted in different amounts of data collected on each animal from those 184 hours of observation. The recorded data, in hours per animal, per treatment, where n = total (baseline, treatment, post-treatment) are presented in Table 4.

Observations during the baseline and post-treatment conditions were conducted both indoors and outdoors. Behaviors listed in the ethogram were observed and recorded much less frequently during outdoor observations than during indoor observations. This was likely due to the additional area in the outdoor enclosure compared to the indoor enclosure, providing the gorillas with more room to spread out, resulting in less potential for interaction. When averaging three hours of observation per day for the baseline and post-treatment conditions, 67% of the data derived from outdoor observations. When comparing baseline and post-treatment averages to the treatment condition average, in which behaviors were collected 100% indoors, the significantly lower rates of behavior recorded during the outdoor observations disproportionately lowered the baseline and post-treatment averages. For illustration, refer to Figures 1 (rates of behavior separated by observation location) and Figure 2 (average rate of behavior per day, including both indoor and outdoor observations).

Figures 1 and 2 show that averaging data from both indoor and outdoor observations produces a significant difference for active aggression between the

experimental conditions ($Q_{0.05, 5.99} = 9.33$, p=0.009). The low rates of behavior recorded outdoors bring the baseline and post-treatment averages down, presumably causing the treatment condition to appear to have a significantly higher rate of aggressive behavior. To correct for this disproportion of behavioral rates between indoor and outdoor exhibits, data from outdoor observations were excluded from analysis and only indoor observations were used, as indoor observations were present in all three experimental conditions. A comparison of the corrected averages (indoor data only) still produced a significant difference for Active Aggression between the experimental conditions ($Q_{0.05, 5.99} = 7.00$, p=0.030).

Critically examining the behavioral data further, the baseline and post-treatment conditions each only had one hour of indoor data collection. One cannot safely assume that behavior rates in the mid-morning and noon observation periods would be equal to those observed during early morning observations. To correct for the potential confound of the varying times of data collection, only the first hour of data collected indoors each day were used in analysis. After removal of outdoor observations and indoor 2$^{\text{nd}}$ and 3$^{\text{rd}}$ hour observations, the final subset of collected data used for analysis is presented in Table 4.

A comparison of Active Aggression, using the final sub-set of data (indoor, 1$^{\text{st}}$ hour only), resulted in no significant difference between the experimental conditions. Mean rate of behavior per gorilla, within each experimental condition, is presented in Table 5. Data analysis of all four behavioral categories; Active Aggression ($Q_{0.05, 5.99} = 5.33$, p=0.069), Passive Aggression ($Q_{0.05, 5.99} = 2.80$, p=0.247), Affiliative behavior ($Q_{0.05, 5.99} = 0.33$, p=0.846), and Stereotypic/Solitary behavior ($Q_{0.05, 5.99} = 1.33$, p=0.513)

resulted in a finding of no significant differences in behavior observed between the different experimental conditions for the gorilla group.

Significant observer effect, or Hawthorn effect (Lehner, 1996), occurred during this study. Despite his best efforts to be neutral, the observer was unable to be physically present in the gorilla building without drawing attention, usually negative, from the silverback male (GI). For each hour of observation, GI displayed at him, aggressively and often, for approximately 10-15 minutes before resuming other behaviors. Aggressive displays towards the observer, though less frequent, occurred sporadically throughout the remainder of each observation. Each aggressive display towards the observer was scored as aggression towards OB (observer). Analysis of the behavioral initiator/receiver data showed almost eight times (8x) more aggression from GI towards OB than towards any of the gorillas within the group (Figure 3). Approximately 75% of all active aggression (271 of 361 occurrences) from GI was directed towards the observer.

Cortisol Data

For each gorilla from which urine was collected, concentration of cortisol in the urine samples, corrected for creatinine, was plotted to observe overall trends. As shown graphically in Figures 4-7, cortisol levels appear to increase over the course of the study for each animal. Best-fit line regressions showed a positive slope from the beginning to the end of the study period in each case.

Observed range of cortisol concentrations (low, high, and mean) for each gorilla during each experimental condition are presented in Table 6. Analysis of low, high, and mean cortisol concentrations, using Friedman's non-parametric ANOVA, for each treatment condition resulted in a statistically significant difference in the low

concentrations ($Q_{0.05,\ 5.99}$ = 6.50, p=0.039) and in the mean concentrations ($Q_{0.05,\ 5.99}$ = 8.00, p=0.018) among the experimental conditions. A Bonferroni post hoc test indicated the post-treatment cortisol concentrations were significantly higher than the baseline cortisol concentrations in both low and mean concentrations. No significant difference was observed when comparing high concentration values from each animal between the treatment conditions. Descriptive and statistical analyses indicate that cortisol levels gradually elevated throughout the course of this study. In fact, low and mean cortisol values increased for each animal from baseline to treatment condition and again from treatment to post-treatment condition (Table 6, Figure 8).

"Fortuitous" measures of physiological relevance in the gorilla urinary cortisol EIA

Animal keepers continued collection of urine samples from the gorillas, though less frequently, after the study ended, in an effort to maintain the trained behavior of providing urine samples on request. Additional samples collected through the end of June 2002 were also analyzed for cortisol, though none of these results from samples collected outside the limits of this study were included in statistical analysis. Interestingly, these post-study samples from one gorilla, GI, contained extremely high cortisol concentrations, and showed a pattern of exponential increase. No samples for GI were available past 6/22/02, so we were unable to determine the apex of this peak, or when the trend returned to more normal levels. For the additional samples, the highest cortisol concentration observed was 664.72 ng/mg cr. For comparison, the highest cortisol concentration observed for GI at any point during the study was 215.23 ng/mg cr; more than three times less than that observed on 6/22/02. The elevated cortisol

concentrations, compared to the elevations during the study period, are presented in Figure 9.

Behavioral and Cortisol Correlation

Spearman's rank test (r, $p < 0.05$) was used to examine the potential relationship between the four behavioral categories and cortisol concentrations from urine samples. Analysis was performed for each individual gorilla. No significant correlations resulted. Results of Spearman's rank test are presented in Table 7.

DISCUSSION

Behavior

Results from this study suggest that there were no significant behavioral indicators of stress during one month of indoor confinement. The gorilla group did not display behavioral changes which would indicate a significant rise in stress during the period of indoor confinement. Lack of significant change in any behavioral category examined, as well as a lack of correlation between behavioral categories and cortisol concentration, indicates that restriction of the gorilla group to their indoor holding facilities for a period of one month may not have elicited a behavioral response to the potential stressor, as had been predicted.

A factor which potentially confounded the expected behavioral results was the silverback male gorilla's reaction and continued aggressive response to the observer. GI's focus on the observer increased over the course of the study. Initially, aggressive displays towards the observer occurred only in the gorilla indoor holding facility. As the study progressed, GI began to display at the observer while outside, having recognized the observer among the various park guests. Such focus on an individual external to his group was unusual, and obviously altered not only his behavior, but possibly the behavior of the other members of the gorilla group, as they reacted to his abnormally aggressive state. Making conclusions regarding the frequency of aggressive or affiliative behavior during the study is more difficult due to this large observer effect.

Cortisol

While behavioral data did not appear to significantly change over the course of the study, comparison of cortisol concentrations across the three treatment conditions indicated an increasing trend from baseline to treatment condition as well as from treatment to post-treatment condition, and a statistically significant difference between the baseline and the post-treatment conditions. It is difficult to attribute a cause for this increase. While the increase from baseline phase to treatment phase may have been due to the space restriction, as predicted, the increase may also have been due to the presence of the observer, and GI's continued aggression towards him. Cortisol increases from treatment phase to post-treatment phase are even more difficult to interpret. The elevated cortisol levels may have resulted from the novelty of a return to the outdoor exhibit and the refurbished condition of that outdoor environment, the continued presence of the observer, or a combination of both.

While the increase in cortisol concentrations throughout the course of the study does represent a statistically significant trend, it does not necessarily equate to a physiologically significant cortisol increase that would be indicative of a negative stress response or decreased animal welfare. In order to understand fluctuating levels of cortisol in gorillas, these data were compared to known urinary cortisol values in gorilla from previous studies. This comparison provides a framework in which to interpret the results of this study.

In four previous studies, baseline urinary cortisol values for captive lowland gorilla have been reported. Czekala et al. (1994) reported range from approximately 10 – 140 ng/mg cr with a mean of 63.0±7.9 ng/mg cr in captive lowland gorillas, and range

from approximately 10 – 80 ng/mg cr, mean = 26.7±4.1 ng/mg cr in wild mountain gorillas. Robbins and Czekala (1997) reported means ranging from approximately 550 – 650 ng/mg cr varying by dominance rank and approximately 600 – 850 ng/mg cr varying by age class in wild male mountain gorillas (no hard values reported in text; means interpreted from figures). Czekala and Robbins (2001) reported means ranging from 550 – 800 ng/mg cr varying by age class in wild male mountain gorillas (no hard values reported in text; means interpreted from figures). Stoinski et al. (2002) reported means ranging from 98.77 – 435.85±66.84 ng/mg cr varying by age class and social group in captive lowland gorillas.

One previous study experimentally observed cortisol levels and, potential stress, under varying conditions. Bahr et al. (1998) examined cortisol levels and stress in captive female lowland gorillas, related with the stressor of giving birth. This study reported a range from 540 – 730 ng/mg cr with a mean of 620±10 ng/mg cr in pregnant (14 - 1 day prepartum) females and a range from 270-510 ng/mg cr with a mean of 380±20 ng/mg cr in new mothers (1 - 14 days post-partum) (original data reported in µg/mg cr, but was converted by this author for consistent comparison with other reported values and the values reported in this study). This study concluded that there was a significant statistical and physiological difference in cortisol levels between pre- and post-partum females. As a benchmark for physiologically significant differences in urinary cortisol values in captive lowland gorillas, the mean for the post-condition was nearly 2x lower (1.63x) for the pre-condition.

Values in the present study ranged from 4.82 – 351.52 ng/mg cr, with means ranging from a low of 63.27±7.37 ng/mg cr in the baseline condition (BE) to a high of

252.72±43.01 ng/mg cr in the post-treatment condition (HO). Per animal, the mean

increases from the baseline condition to the post-treatment condition were as follows:

GI: 82.76±7.02 to 142.06±8.86 ng/mg cr; increase = **1.72x**
BE: 63.27±7.37 to 145.11±23.23 ng/mg cr; increase = **2.29x**
HO: 164.5±14.55 to 252.72±43.01 ng/mg cr; increase = **1.54x**
HA: 110.21±15.43 to 158.97±43.07 ng/mg cr; increase = **1.44x**

Observed cortisol concentrations for each animal, during each treatment condition of this

study, were all within the previously reported range of baseline cortisol concentrations in

captive western lowland gorillas. That the urinary cortisol values of this gorilla group are

within the normal baseline ranges of previously measured urinary cortisol ranges in other

gorillas indicates that the individuals in this group may not have experienced any acute

stressors which elicited a strong physiological stress response during the course of this

study.   This absence of a physiologically significant acute stressor during the study is

further illustrated when the cortisol values during the study are compared to the higher

cortisol values observed in response to a known acute stressor (illness) after the study

ended. Despite this lack of any demonstrative acute stressor during the study, this does

not preclude the potential for the gorillas to have experienced a physiologically

significant chronic stress in response to the continual presence of stressors, namely

indoor confinement, observer effect, and a modified outdoor environment.   The

magnitude of cortisol increase over baseline conditions through the course of this study is

consistent with the magnitude of cortisol increase previously reported as physiologically

significant (Bahr, et al. 1998). Therefore, the increase in cortisol observed during this

study was a physiologically significant increase. The observed cortisol increase was not

a response to an acute stressor, but more likely, the response to one or more chronic

changes to the gorillas' routine environment.

<u>Space Restriction</u>

Behavioral observations during this study did not support the gorilla group employing either of the social coping strategies used by other primates to reduce the potential for agonistic interactions during periods of space restriction or increased density. Under short-term space restrictions, captive chimpanzees (Aureli and de Waal, 1997), macaques (Aureli et al, 1995), and squirrel monkeys (Perloe, 1986) each displayed a conflict avoidance strategy, aimed at reducing the risk of aggression by decreasing the overall level of movement and social activity. Use of this strategy by these primates resulted in an observed reduction in affiliative behaviors and an increase in submissive behaviors. Under longer-term space restrictions, rhesus monkeys (Judge and de Waal, 1997) and bonobos (Sannen, et al. 2004) have been observed to utilize a more active tension-reduction strategy, such as increased affiliative allogrooming, which functions to relieve tension due to higher densities, and thus lower the potential for aggression. Affiliative behaviors neither decreased nor increased significantly during this study. With the effect of both strategies to lower aggression, a decrease in aggression would have been expected had either strategy been utilized by the gorillas during this study. Active Aggression, though not statistically significant in the group, actually increased for three of the four adult gorillas (BE, HO, and HA) during the space restriction. Average cortisol levels increased steadily through the duration of the study. While the causal factor(s) of this increase in unclear, it is not likely to be the result of active repression of agonistic behaviors, as has been observed in chimpanzees (de Waal, et al. 2000), because tight social bonds between group members are not observed in gorillas as they are with

chimpanzees. Therefore, gorillas are less likely to expend significant effort to preserve social relationships.

From the results of only one study, it would be premature to conclude that gorillas differ from other primates studied in their response to space restriction, but it would not be completely unexpected if, in fact, that were the case. Gorillas differ from the other primates studied in that they are have a polygamous mating system in which strong social bonds between the members of the group, particularly between female members, are not critical to the group stability, and affiliative interactions are rare (Stokes, 2004; Stokes and Parnell, 2002). More important to the stability of a gorilla group, is a strong silverback male to protect against infanticide against other adult males (Stokes, 2004). Since gorilla females do not develop substantial social relationships among one another, maintain primary proximity, though limited interaction with the group's silverback, and transfer between groups with only little resistance (Stokes and Parnell, 2002), they would be less likely to actively strive to reduce social tension caused by agonistic encounters during times of space restriction or high density.

CONCLUSION

While the gorillas did appear to react physiologically to one or more chronic stressors during the course of this study, the stress experienced was not severe, and likely had little to no impact on their overall animal welfare. Framing this stress in terms of Moberg's (2000) model of stress, the gorillas perceived a stressor during the study period. In response to that stressor, a neuroendocrine response to that stressor was initiated, as evident by the increased levels of cortisol observed. The stressor, however, was minor; as temporary indoor confinement, novelty of a new exhibit, or observer effect is a much less serious threat to homeostasis than more harmful stressors, such as illness or injury. The stress response, consistent with the magnitude of the stressor, was also minor. The gorillas' neuroendocrine response to the minor stressor was sufficient to address the stressor without compromising normal biological functions, preventing the stress response from progressing to a pre-pathological or pathologic state. With normal biological function maintained, the biological impact of this stressor on the animal welfare of the gorillas could be considered negligible.

The gorillas' lack of observed negative stress-response to the potentially stressful space restriction during this study may be explained by the additional enrichment provided to the gorillas during their indoor confinement. Keeper staff provided additional browse, more enrichment items, additional training sessions, and increased keeper interaction while the gorillas remained indoors. These animal care and management techniques may have buffered the predicted increases in stress by providing stimulating novelty in the gorillas' indoor environment. This assumption is consistent

with the findings of Boinski, et al. (1999), in which increased quantity and complexity of environmental enrichment was demonstrated to reduce stereotypic behaviors and lower cortisol concentrations.

This study contributes to the growing body of literature regarding zoo animal welfare. Through a multi-faceted analysis of a potentially stressful situation, we learned more about the stress response of a gorilla group temporarily confined to their indoor enclosure. Further research would be required, however, before the results of this study could be generalized to the captive gorilla population. To obtain a better understanding for how gorillas respond to this condition, a multi-institution zoo study, examining behavior and cortisol, is recommended. Results of such a study would have great potential to reach appropriate and applicable conclusions based on a larger sampling of the captive gorilla population, and potentially provide animal management suggestions regarding the welfare of gorillas while temporarily, or seasonally, confined to indoor enclosures.

Temporary indoor confinement is a reality of animal management in zoological facilities for many species. Zoos which are located in regions which experience cold winter seasons often restrict animal access to outdoor enclosures, dependant upon outdoor temperatures. This seasonal space restriction is a potential stressor that many zoo animals face each year. Careful examination of how various animal species perceive this potentially stressful situation, through multiple measures of stress, can be a valuable tool to animal care personnel, as the strive to provide optimal animal welfare for the animals in their care.

Table 1. History of a group of western lowland gorillas at Disney's Animal Kingdom.

Name	Gender	Birth Date	Weight (at beginning of study)	Zoo of Origin	Sire/Dam History
GI	Male	12/30/1980	452 lbs.	Lincoln Park Zoo, Chicago (born in Rotterdam, Netherlands)	Ernst/Salome (both wild-caught)
BE	Female	4/21/1971	255 lbs.	Lincoln Park Zoo, Chicago	Kisoro/Helen (both wild-caught)
HA	Male	10/12/1994	156 lbs.	Lincoln Park Zoo, Chicago	GI/BE
MK	Female	1/24/1999	42 lbs.	Disney's Animal Kingdom	GI/BE
HO	Female	9/7/1983	178 lbs.	Lincoln Park Zoo, Chicago	Koundu/Kisuma (both captive born)
JA	Male	11/4/1997	67 lbs.	Disney's Animal Kingdom	GI/HO

Table 2. Housing schedule of a group of western lowland gorillas at Disney's Animal Kingdom throughout the course of the study.

	Baseline Feb. 25-Mar. 24, Apr. 7-8, 2002	**Baseline** Mar. 25–Apr. 6, 2002	**Treatment** Apr. 9-May 6, 2002	**Post-treatment** May 7-June 2, 2002
Park opens	09:00	08:00	09:00	09:00
Group shifted outside	09:00	08:00	N/A	09:00
Group shifted to bachelor side for cleaning	N/A	N/A	09:15-09:30 or 10:30-10:45	N/A
Group shifted inside	17:00	18:00	N/A	17:00

Table 3. Behavioral data collection schedule

	Baseline Feb. 25-Mar. 24, Apr. 7-8, 2002	**Baseline** Mar. 25–Apr. 6, 2002	**Treatment** Apr. 9-May 6, 2002	**Post-treatment** May 7-June 2, 2002
1st observation (1hr w/in timeframe)	Indoors-07:45-09:00	Indoors-06:45-08:00	08:00-09:00	Indoors-07:45-09:00
2nd observation (1hr w/in timeframe)	Outdoors-09:00-10:30	Outdoors-09:00-10:30	9:30-10:30	Outdoors-09:00-10:30
3rd observation (1hr w/in timeframe)	Outdoors-10:30-12:00	Outdoors-10:30-12:00	11:00-12:00	Outdoors – 10:30-12:00

Table 4. Hours of behavioral data collected over the duration of the study; Total hours and hours from only the 1st observation period.

	GI		BE		HO		HA		JA		MK	
	Total	1st hr	Total	1st hr	Total	1st hr	Total	1st hr	Total	1st hr	Total	1st hr
Baseline	78	28	66	26	62	22	77	28	74	27	71	26
Treatment	55	20	53	17	55	20	56	20	56	20	45	13
Post-treatment	41	16	37	14	40	12	46	16	45	14	44	12

Table 5. Mean rates of behavioral within each experimental condition for each gorilla in the family group at Disney's Animal Kingdom. Values represent mean ± SEM.

Subject	GI			BE			HO		
Treatment	**Pre**	**Treat**	**Post**	**Pre**	**Treat**	**Post**	**Pre**	**Treat**	**Post**
Active Aggression	8.23±0.68	6.95±1.06	6.56±0.70	0.23±0.10	0.57±0.26	0.08±0.07	0.55±0.27	2.67±0.66	1.42±0.47
Passive Aggression	3.25±0.42	2.98±0.53	1.60±0.34	0.31±0.14	0.37±0.25	0.33±0.18	0.00±0.00	1.48±0.40	1.60±1.01
Affiliative Behavior	0.27±0.12	0.07±0.07	0.25±0.11	0.69±0.32	0.37±0.25	0.41±0.18	0.18±0.14	0.67±0.27	0.33±0.33
Stereotypic/Solitary	1.89±0.37	0.47±0.34	1.08±0.34	1.74±0.31	1.82±0.42	2.62±0.65	1.38±0.59	3.82±0.53	3.17±0.83

Subject	HA			JA			MK		
Treatment	**Pre**	**Treat**	**Post**	**Pre**	**Treat**	**Post**	**Pre**	**Treat**	**Post**
Active Aggression	5.35±0.53	7.95±1.70	5.00±0.63	1.15±0.34	2.17±0.38	1.79±0.39	0.18±0.09	0.15±0.15	0.00±0.00
Passive Aggression	0.54±0.12	1.67±0.36	0.30±0.13	0.04±0.04	2.17±0.31	0.52±0.68	0.00±0.00	0.00±0.00	0.00±0.00
Affiliative Behavior	4.48±0.70	2.00±0.48	3.79±0.84	3.60±0.59	5.08±0.77	3.86±1.00	3.01±0.59	3.26±0.69	3.61±1.21
Stereotypic/Solitary	2.93±0.44	2.53±0.50	2.04±0.41	3.79±0.51	2.98±0.46	2.86±0.74	3.54±0.50	2.39±0.55	1.89±0.66

Table 6. Range (low, high, and mean) of cortisol concentrations (in ng/mg cr) from urine samples, within each experimental condition, for each adult gorilla in the family group at Disney's Animal Kingdom. Mean values represent mean ± SEM.

Subject	Baseline			Treatment			Post-treatment		
	Low	High	Mean	Low	High	Mean	Low	High	Mean
GI	33.34	148.43	82.76±7.02	76.99	215.23	139.46±8.62	59.09	183.82	142.06±8.86
BE	23.12	151.86	63.27±7.37	38.88	149.03	90.09±14.72	85.08	252.68	145.11±23.23
HO	27.43	241.78	164.5±14.55	126.21	318.98	202.43±16.88	141.94	351.52	252.72±43.01
HA	4.82	308.45	110.21±15.43	12.43	223.60	130.5±13.12	53.11	177.38	158.97±43.07

Table 7. Correlation (Spearman's r, p<0.05) between rates of behavior and cortisol concentrations for each adult gorilla in the family group at Disney's Animal Kingdom.

	Active Aggression	Passive Aggression	Affiliative Behavior	Stereotypic/ Solitary Behavior
GI	-0.11, p=0.54	-0.05, p=0.80	0.09, p=0.61	-0.19, p=0.29
BE	-0.24, p=0.37	-0.43, p=0.08	0.41, p=0.09	0.09, p=0.73
HO	-0.49, p=0.11	-0.36, p=0.25	-0.01, p=0.99	-0.27, p=0.40
HA	0.06, p=0.74	-0.10, p=0.58	-0.20, p=0.27	-0.29, p=0.12
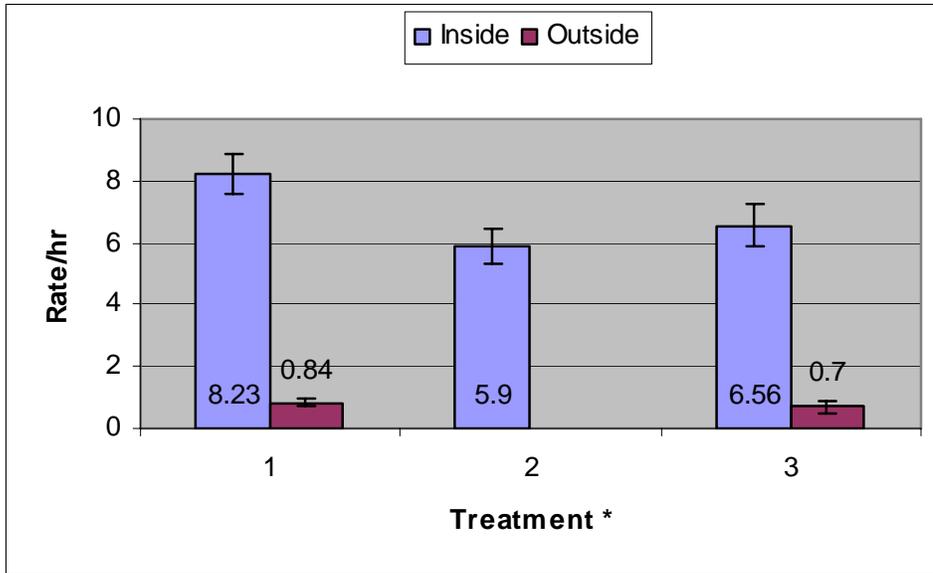
Figure 1. Indoor and Outdoor rates of active aggression for a male silverback gorilla at Disney's Animal Kingdom. Values represent mean ± SEM. * (1 = Baseline, 2 = Treatment, 3 = Post-treatment)
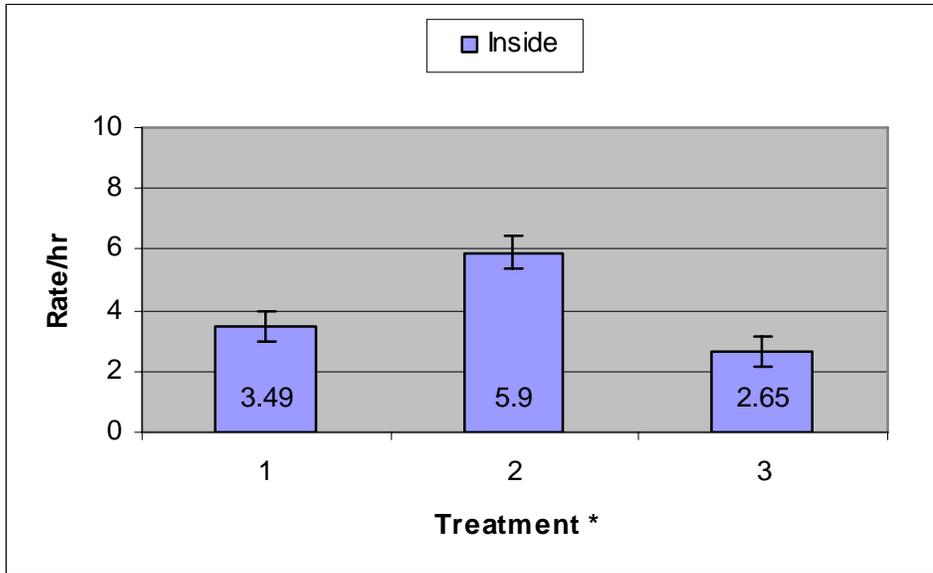
Figure 2. Average rates of active aggression for a male silverback gorilla at Disney's Animal Kingdom. Values represent mean ± SEM. * (1 = Baseline, 2 = Treatment, 3 = Post-treatment)
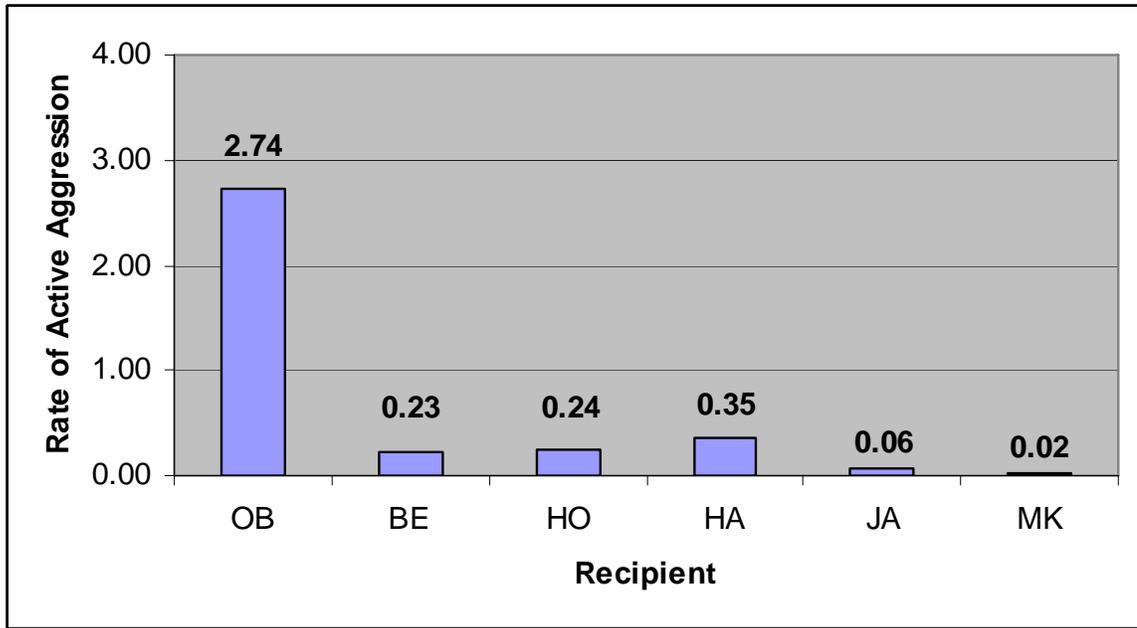
Figure 3. Rates of active aggression directed to various recipients from a male silverback gorilla at Disney's Animal Kingdom.
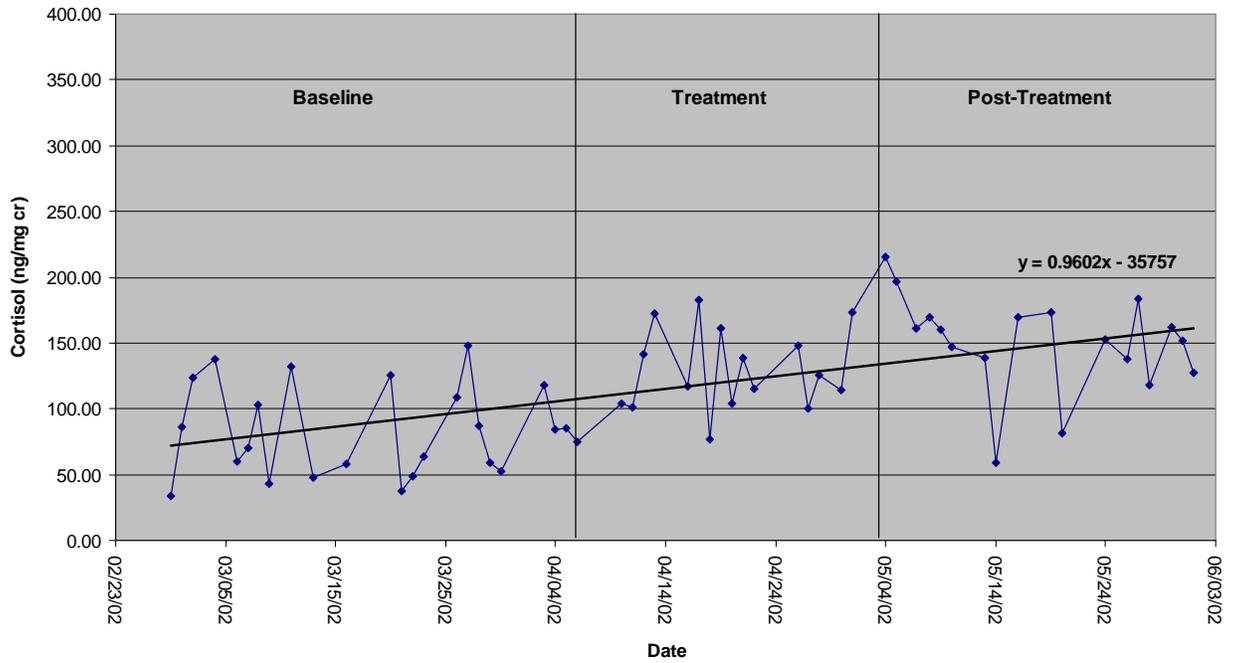
Figure 4. Cortisol concentrations in urine samples from a male silverback gorilla (GI) at
Disney's Animal Kingdom, throughout the course of the study. Values presented in
ng/mg cr.
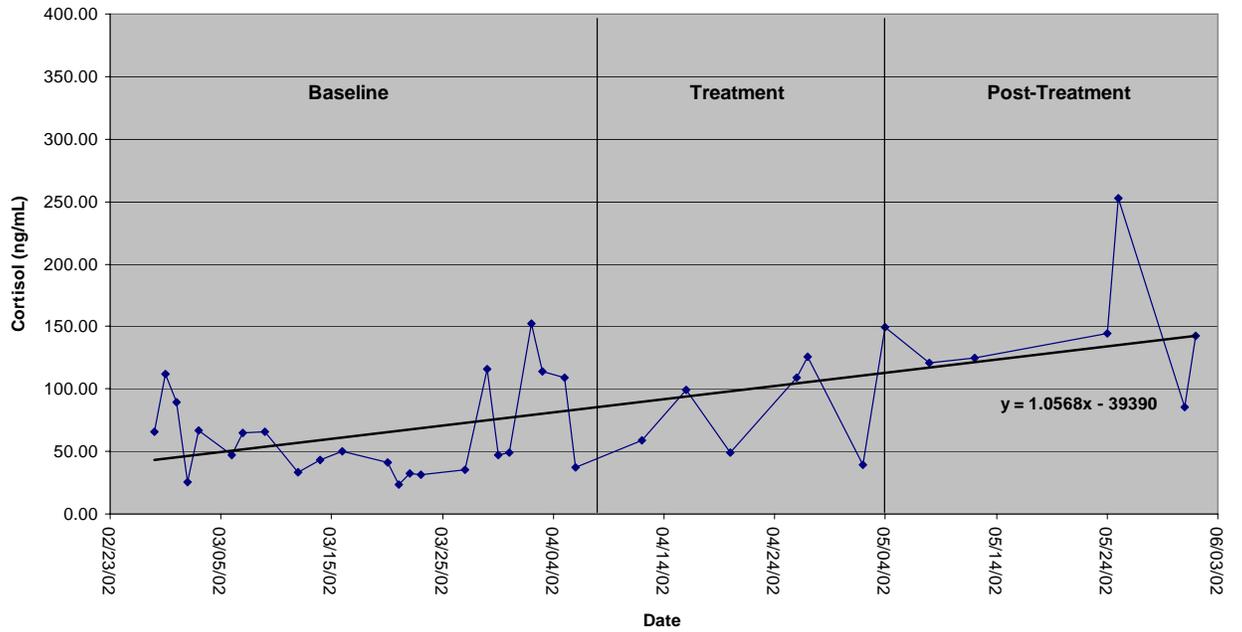
Figure 5. Cortisol concentrations in urine samples from an adult female gorilla (BE) at Disney's Animal Kingdom, throughout the course of the study. Values presented in ng/mg cr.
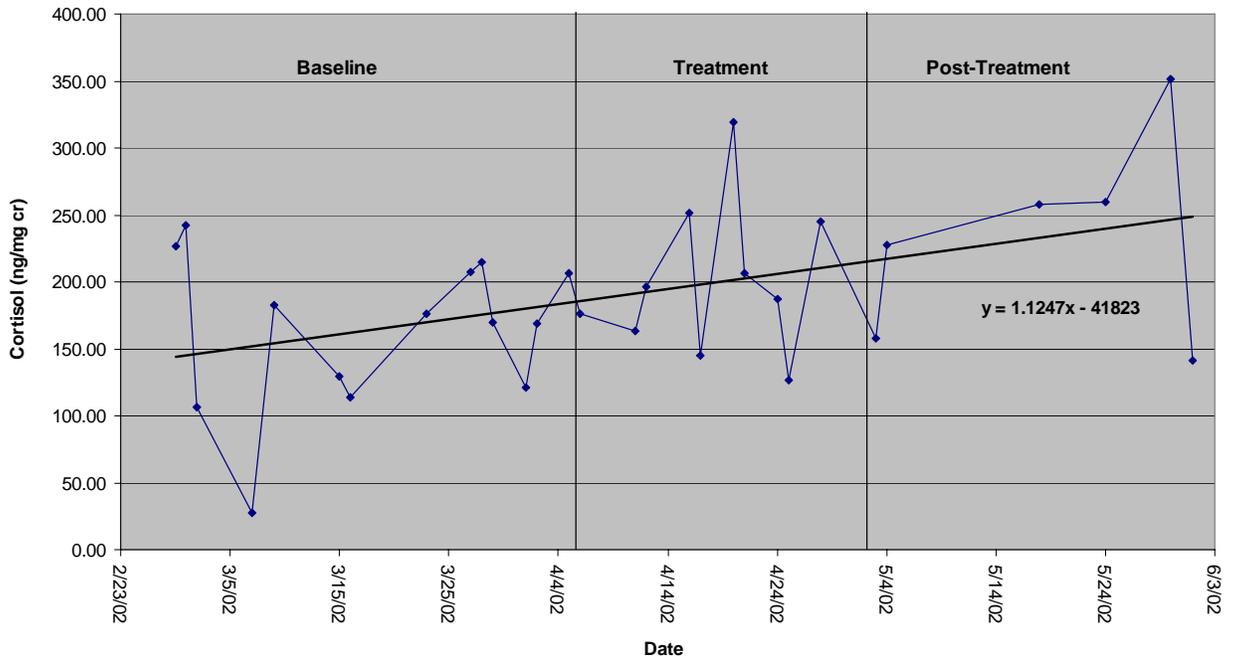
Figure 6. Cortisol concentrations in urine samples from an adult female gorilla (HO) at Disney's Animal Kingdom, throughout the course of the study. Values presented in ng/mg cr.
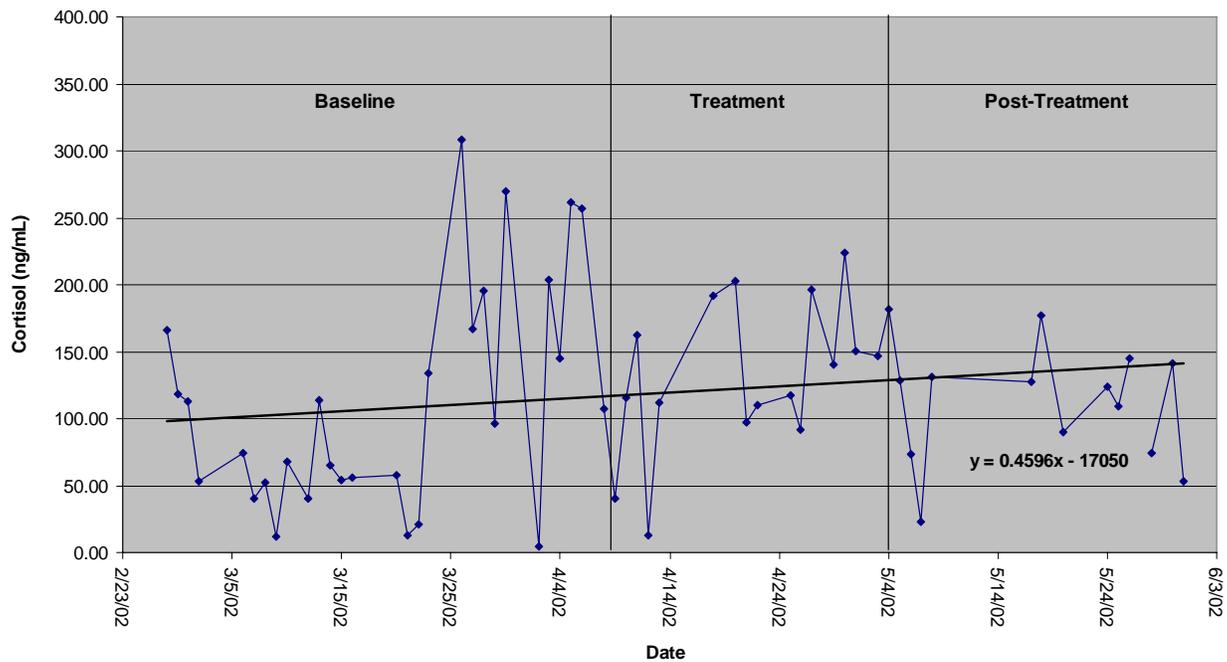
Figure 7. Cortisol concentrations in urine samples from a blackback male (HA) at Disney's Animal Kingdom, throughout the course of the study. Values presented in ng/mg cr.
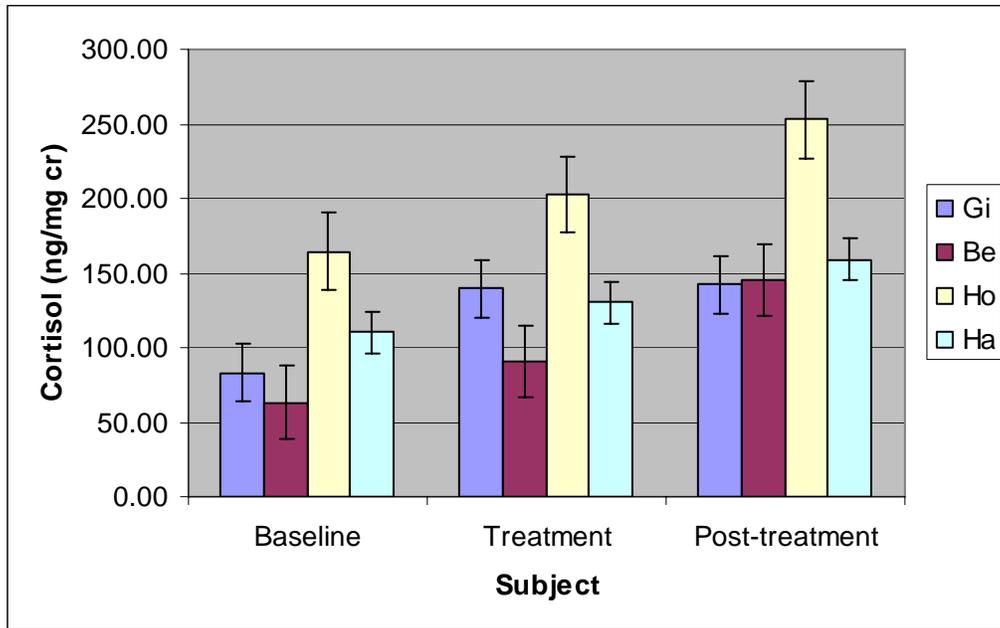
Figure 8. Cortisol concentrations in urine samples from each adult gorilla during each experimental condition. Values represent mean ± SEM.
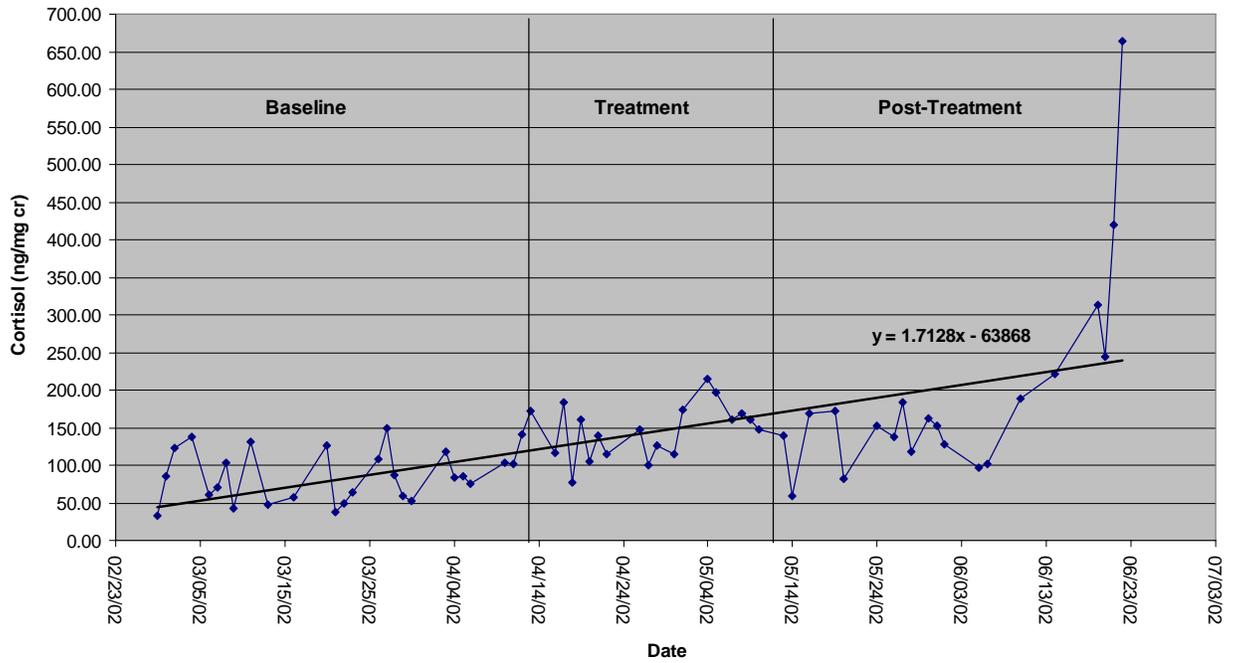
Figure 9. Cortisol concentrations from a male silverback gorilla (GI) at Disney's Animal Kingdom, including urine samples throughout the course of the study and available urine samples after the study ended. Values presented in ng/mg cr.

APPENDIX A: BEHAVIORAL ETHOGRAM

Ethogram for monitoring interactions in a group of gorillas

This ethogram was developed through use of behavioral definitions from many different ethograms presented in *Compilation of Gorilla Ethograms*, compiled by the Gorilla Behavioral Advisory Group, affiliated with the Gorilla Species Survival Plan (SSP), January 1991.

All Occurrences Behaviors: All occurrences of the following behaviors were recorded for all animals.

Thee one-hour observations occurred between the hours of 6:45 and 12:00, five days per week. Each observation was one hour in duration. The one-hour observations were divided into four 15-minute quarters. If any animal was not visible for the duration of any quarter, Not Visible (NV) was scored for that quarter. If an animal was visible for all or part of any quarter, regardless of whether or not behaviors from the ethogram were performed, the animal was not scored NV.

For behaviors that occurred as a state, rather than an event, continuous occurrence of those behaviors were counted as one bout regardless of the duration. If there was a gap of five or more seconds between bouts, then it was scored as a separate occurrence of the behavior. Here after, this is referred to as the 5-second rule.

Active Aggression

Behavior	**Definition**
Chase	One animal moves in pursuit of another who is rapidly moving away; usually in a running gait.
Chest Beat	Rapid, rhythmical striking of the chest or trunk with slightly cupped hands. Usually performed bi-pedally; either standing stationary, walking, or charging.
Charge	Animal rushes up to or past another individual in a short, running burst, either quadrapedally or bi-pedally.
Object Slap	Slapping an object in the environment with a hand or foot, or slamming the shoulder or side of body into an object such as a shift door, vegetation, etc. Usually follows a charge.
Hit	Animal strikes another individual with hand or foot.
Bite	Animal seizes a part of the body of another individual with its teeth during an aggressive interaction.

Grab Object	One animal grasps or snatches an object (food, browse, or otherwise) from another individual.
Throw/Flail Object	Object (can include branches, rocks, dirt, feces, enrichment items, etc.) is thrown or struck with enough force to propel that object. Often oriented towards an individual.

Passive Aggression

Behavior	**Definition**
Threaten	Physical gestures that accompany a threatening vocalization. Head lunge, mouth moderately open, lips in a hooting shape. Directed from one individual to another.
Tight Lip Stare	Head slightly tipped downward, eyes hard and fixed on an individual, brow furrowed. Lips pressed together tightly and/or curled back over teeth (5 second rule).
Stiff Stance	Animal has a rigid/stiff, quadrapedal posture with elbows and shoulders tight, standing still. Often associated with Tight Lip Stare (5 second rule).
Displace	One animal approaches conspecific whom then moves away. May be used to assume the position of the latter or to prevent the latter access to that particular area.
Open-Mouth Threat	A tense facial expression with mouth open and lips raised, exposing the canines to another individual.

Affiliative Behaviors

Behavior	**Definition**
Contact	An animal contacts a conspecific in a manner not previously described as aggressive.
Approach	An animal moves to within one arm length of another, and stays within one arm's length for more than 5 seconds.
Nurse	An individual suckles the breast of its mother or other adult female.
Carry	One individual lifts up and carries another individual.

Ride	An individual rides on, usually on the back of, another individual.
Social Play	Play behaviors performed with conspecifics. May have both gentle and aggressive components. Includes wrestling, tickling, biting, laughing, chasing, hit and run away (often accompanied by chasing), chest beat, object or ground slap, and/or many other possibilities (5 second rule).
Initiate Social Play	Social play is attempted by one individual towards another, but the play is not reciprocated.
Social Grooming	One animal manipulates the fur, extremity, or orifice of another animal (5 second rule).
Socio-sexual	Behavior, between two individuals, that is sexual in nature. Mounting another individual, copulation, manipulation of another's genitalia with hands, feet, or mouth, or masturbation are examples. (5 second rule).

Stereotypic/Solitary Behavior

Behavior	**Definition**
Rocking	Repetitive movement of the head and trunk, either back and forth or side to side (5 second rule).
Regurgitation/Reingestion	Food is brought up from the stomach to the mouth and then re-ingested.
Ear-Clasp	Embracing or use of hands to hold onto one's own ears, often cupping the entire external ear pinnae (5-second rule).
Huddle	Two or more inactive individuals with torsos in direct contact. Arms may be wrapped around one another (5-second rule).
Coprophagy	Ingestion of fecal material.
Other Stereotypic	Other behaviors not previously defined exhibiting excessive repetition of or lack of variation in movements, postures, or patterns of travel (5 second rule).
Self Play	An animal performs play behaviors without the participation of a conspecific.

Self Groom	An animal grooms its own body

Other Behaviors (Submissive)

Behavior	**Definition**
Crouch	Animal hunches down by lowering head, hunching shoulders, and often covering head with arms; often a response to a threat or attack of another individual
Avoid	An animal moves out of the path of an approaching animal or takes a less direct route around that animal.

APPENDIX B: CREATININE ASSAY

<u>**Creatinine Assay Methods and Details**</u>

DAK WTC CREATININE ASSAY PROTOCOL

1.   **Pipet 100µl of standards, controls, and samples onto Micro titer plates**
       -    <u>Standards</u>: 0.0005, 0.0009, 0.0019, 0.0038, 0.0075, 0.015, 0.030 mg/50µl
            creatinine
       -    <u>Controls</u>: C1 = 0.01 and C2 = 0.005 mg/50µl *

            * C2 used in this study was 0.001 mg/50µl, which varied from the
            previously established DAK WTC protocol.

2.   **Dispense 50µl 0.75M NaOH to each well**

3.   **Dispense 50µl 0.04M Picric Acid into all wells**

4.   **Incubation**
       -    Incubate assay at room temperature for 5-10 minutes

5.   **Read Optical Density (OD) on the Molecular Devices Emax plate reader
       (model E9996)**
                 Absorbance Measures:
                 -    Measuring filter: 490nm
                 -    Reference filter: 650nm

6.   **Enter OD #'s into Excel regression calculation spreadsheet to calculate
       creatinine results (mg/50µl)**

7.   **Multiply creatinine results (mg/50µl) by dilution of the sample for final
       creatinine value (mg/ml)**

<u>Applying DAK WTC Creatinine Assay Protocol to Gorilla Urine</u>

    Test assays using randomly selected unknown samples were run to determine the

most appropriate dilution to run the majority of the samples. A sample dilution of 1:50

resulted in the majority of the test samples registering an OD which read between 20%

and 80% on the standard curve. All samples were then initially assayed at 1:50. Any

samples which registered an OD of less than 0.310 (sample too dilute) or 1.200 (sample

too concentrate) had ranges outside of what could be reliably determined against the standard curve. These samples were diluted more, or less, as appropriate, and re-assayed until the resulting OD was within the reliable range of the standard curve (20-80%). Dilutions used varied among the individual gorillas, as GI and HO had very concentrated urine, and required extensive dilution for their samples' OD to fit on the standard curve (1:50 to 1:300). Conversely, BE and HA had more dilute urine, which did not need to be further diluted to the extent of GI and HO. BE and HA dilutions ranged from 1:50 to 1:100.

Data obtained ranged from 0.100 to 1.745 Cr mg/ml. Average results (± STD err) for each animal were as follows: GI – 0.80 ± 0.03 Cr mg/ml; BE – 0.72 ± 0.05 Cr mg/ml; HO – 0.96 ± 0.06 Cr mg/ml; and HA – 0.37 ± 0.03 Cr mg/ml.

LIST OF REFERENCES

Abbott, D.H., Keverne, E.B., Berchovitch, F.B., Shively, C.A., Mendoza, S.P., Saltzman, W., Snowden, C.T., Ziegler, T.E., Banjevic, M., Garland. T., Jr., and Sapolsky, R.M. 2003. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior. 43: 67-82.

Akers, J.S. and Schildkraut, D.S. 1985. Regurgitation/reingestion and coprophagy in captive gorillas. Zoo Biology. 4: 99-109.

Altmann, J. 1974. Observational study of behavior: sampling methods. Behaviour. 49: 227-267.

Aureli, F. 1991. Clashes of interests, agonistic conflicts and social homeostasis in non-human primates. Rivista di Anthropologica (Roma). 69: 17-25.

Aureli, F., van Panthaleon van Eck, C.J., and Veenema, H.C. 1995. Long-tailed macaques avoid conflicts during short-term crowding. Aggressive Behavior. 21: 113-122.

Aureli, F. and de Waal, F.B.M. 1997. Inhibition of social behavior in chimpanzees under high density conditions. American Journal of Primatology. 41: 213-228.

Bahr, N.I., Pryce, C.R., Döbeli, M. and Martin, R.D. 1998. Evidence from urinary cortisol that maternal behavior is related to stress in gorillas. Physiology and Behavior. 64: 429-437.

Bahr N. I., Palme R., Möhle U., Hodges J. K. and Heistermann M. 2000. Comparative aspects of the metabolism and excretion of cortisol in three individual nonhuman primates. General and Comparative Endocrinology. 117: 427-468.

Banks, E.M. 1982. Behavioral research to answer questions about animal welfare. Journal of Animal Science. 54(2):435-446.

Barnett, J.L. and P.H. Hemsworth. 1990. The validity of physiological and behavioral measures of animal welfare. Applied Animal Behaviour Science. 25:177-187.

Bercovitch, F.B. and Clarke, A.S. 1995. Dominance rank, cortisol concentrations, and reproductive maturation in male rhesus macaques. Physiology and Behavior. 58: 215-221.

Boinski, S., Swing. S.P., Gross, T.S., and Davis, J.K. Environmental enrichment of brown capuchins (*Cebus paella*): behavioral and plasma and fecal cortisol measures of effectiveness. American Journal of Primatology. 48: 49-68.

Bowen, R.A. 1980. The behavior of three hand-reared lowland gorillas, *Gorilla gorilla gorilla*, with emphasis on the response to a change in accommodation. Dodo, Journal of Jersey Wildlife Preservation Trust. 17: 63-79.

Broom, D.M. 1991. Assessing welfare and suffering. Behavioural Processes. 25:117-123.

Buchanan, K.L. and Goldsmith, A.R. 2004. Noninvasive endocrine data for behavioural studies: the importance of validation. Animal Behaviour. 67: 183-185.

Buckingham, J.C. 2000. Effects of Stress on Glucocorticoids. In: *Encyclopedia of Stress*. G. Fink (ed.). Academic Press. London, 2: 229-237.

Burks, K.D., Bloomsmith, M.A. Forthman, D.L., and Maple, T.L. 2001. Managing the socialization of an adult male gorilla (*Gorilla gorilla gorilla*) with a history of social deprivation. Zoo Biology. 20: 347-358.

Calhoun, J.B. 1962. Population density and social pathology. Scientific American. 206: 139-148.

Carlstead, K., Brown, J.L., Monfort, S.L., Killens, R., and Wildt, D.E. 1992. Urinary monitoring of adrenal responses to psychological stressors in domestic and non-domestic felids. Zoo Biology. 11: 165-176.

Carlstead, K., Fraser, J., Bennett, C., and Kleinman, D.G. 1999. Black rhinoceros (*Diceros bicornis*) in U.S. zoos: II. Behavior, breeding success, and mortality in relation to housing facilities. Zoo Biology. 18: 35-52.

Carlstead, K., and Shepherdson, D.J. 2000. Alleviating stress in zoo animals with environmental enrichment. In: *The Biology of Animal Stress*. Moberg, G.P. and Mench, J.A. (eds.) CABI Publishing; Cambridge. 337-354.

Clark, J.D., Rager, D.R. & Calpin, J.P. 1997. Animal well-being IV. Specific assessment criteria. Laboratory Animal Science. 47(6):586-597.

Cook, C.J., Mellor, D.J., Harris, P.J., Ingram, J.R. & Matthews, L.R. (2000). Hands-on and hands-off measurement of stress. In: *The Biology of Animal Stress*. G.P. Moberg & J.A. Mench (eds), CAB International, Cambridge. 123-146.

Czekala, N.M., Lance, V.A., and Sutherland-Smith, M. 1994. Diurnal urinary corticoid excretion in the human and gorilla. American Journal of Primatology. 34: 29-34.

Czekala, N. and Robbins, M.M. 2001. Assessment of reproduction and stress through hormone analysis in gorillas. In: *Mountain gorillas: three decades of research at Karisoke.* M.M. Robbins, P. Sicotte, & K.J. Stewart (eds). Cambridge University Press, Cambridge. 317-339.

Dawkins, M.S. (1988). Behavioural deprivation: a central problem in animal welfare. Applied Animal Behaviour Science. 20:209-225.

Dawkins, M.S. (1990). From an animal's point of view: Motivation, fitness, and animal welfare. Behavioural and Brain Sciences. 13:1-9 & 54-61

de Waal, F.B.M. 1989. The Myth of a Simple Relation Between Space and Aggression in Captive Primates. Zoo Biology Supplement 1: 141-148.

de Waal, F.D.M., Aureli, F., and Judge, P.G. 2000. Coping with crowding. Scientific American. 282(5): 76-81.

Doran, D.M., McNeilage, A., Greer, D., Bocian, C., Mehlman, P., and Shah, N. 2002. Western lowland gorilla diet and resource availability: New evidence, cross-site comparisons, and reflections of indirect sampling methods. American Journal of Primatologists. 58: 91-116.

Duncan, I.J.H. & Petherick, C.J. (1991). The implications of cognitive processes for animal welfare. Journal of Animal Science. 69:5017-5022.

Fraser, D. 1995. Science, values and animal welfare: Exploring the 'inextricable connection'. Animal Welfare. 4: 103–117.

Gatti, S., Levréro, F., Ménard, N., and Gautier-hion, A. 2004. Population and group structure of western lowland gorillas (*Gorilla gorilla gorilla*) at Lokoué, Republic of Congo. American Journal of Primatology. 63: 111-123.

Groves, C. 2001. Primate taxonomy. Washington, DC: Smithsonian Institute Press.

Harcourt, A. H. 1981. Inter-male competition and the reproductive behavior of the great apes. In: *Reproductive Biology of the Great Apes*, E. E. Graham (ed.), Academic Press, New York, 301-318.

Harcourt, A.H., Stewart, K.J. 1984. Gorillas time feeding: aspects of methodology, body size, competition and diet. African Journal of Ecology. 22: 207-215.

Hart, B.L. 1987. Behaviour of sick animals. The Veterinary Clinics of North America. Food Animal Practice, W.B. Saunders, 383-391.

Hoff, M.P., Hoff, K.T., Horton, L.C., and Maple, T.L. Behavioral effects of changing group membership among captive lowland gorillas. Zoo Biology. 15: 383-393.

Judge, P.G., and de Waal, F.B.M. 1997. Rhesus monkey behaviour under diverse population densities: coping with long-term crowding. Animal Behaviour. 54: 643-662.

Kuhar, C.W., Bettinger, T.L., and Laudenslager, M.L. 2005. Salivary cortisol and behavior in an all-male group of western lowland gorillas (*Gorilla gorilla gorilla*). Animal Welfare. 14: 187-193.

Lehner, P.N. 1996. Handbook of ethological methods. 2nd ed. University Press; Cambridge, 672 pp.

Lutz, C., Marinus, L., Chase, W., Meyer, J., and Novak, M. 2003. Self-injurious behavior in male rhesus macaques does not reflect externally directed aggression. Physiology & Behavior. 78: 33-39.

Maggioncalda, A.N., Czekala, N.M., and Sapolsky, R.M. 2002. Male orangutan subadulthood: A new twist on the relationship between chronic stress and development arrest. American Journal of Physical Anthropology. 118: 25-32.

Magliocca, F., Querouil, S., and Gautier-Hion, A. 1999. Population structure and group composition of western lowland gorillas in north-western Republic of Congo. American Journal of Primatology. 48: 1-14.

Maple, T., McManamon, R., and Stevens, E. 1995. Defining the good zoo: Animal care, maintenance, and welfare. In: *Ethics of the Ark: Zoos, animal welfare, and wildlife conservation*. Norton, B.G., Hutchins, M., Stevens, E.F., and Maple, T.L. (eds.), Smithsonian Institute Press, Washington, 219-234.

Martin, P. and Bateson, P. 1993. Measuring Behaviour: an introductory guide. 2nd ed. University Press; Cambridge, 222 pp.

Mason, G. and Meldl, M. 1993. Why is there no simple way of measuring animal welfare? Animal Welfare. 2: 301-319.

McEwen, B.S. 2000. Definitions and concepts of stress. In: *Encyclopedia of Stress*. Vol.3. G. Fink, ed. G. Fink. Academic Press.

Mellen, J.D. 1991. Factors influencing reproductive success in small captive felids (*Felid spp.*): A multiple regression analysis. Zoo Biology. 10: 95-110.

Mellen, J.D. and Sevenich-MacPhee, M. 2001. Philosophy of Environmental Enrichment: Past, Present, and Future. Zoo Biology. 20: 211-226.

Mench, J.A., and Kreger, M.D. 1996. Ethical and welfare issues associated with keeping wild mammals in captivity. In: *Wild mammals in captivity: Principles and*

techniques. Kleiman, D.G., Allen, M.A., Thompson, K.V., and Lumpkin, S. (eds.), University of Chicago Press, Chicago, 5-15.

Moodie, E.M. and Chamove, A.S. 1990. Brief threatening events are beneficial for captive tamarins. Zoo Biology. 9: 275-286.

Moberg, G.P. 1985. Influences of stress on reproduction: measures of well-being. In: *Animal Stress*. Moberg, G.P. (ed). American Physiological Society, Bethesda: 245-267.

Moberg, G.P. 1987. Problems in defining stress and distress in animals. Journal of American Veterinary Medicine Association. 191(10): 1207-1211.

Moberg, G.P. 1999. When does stress become distress? Lab Animal. 28(4): 22-26.

Moberg, G.P. 2000. Biological response to stress: Implications for animal welfare. In: *The Biology of Animal Stress*. Moberg, G.P. and Mench, J.A (eds). New York: CABI Publishing.

Muller, M.N. and Lipson, S.F. 2003. Diurnal patterns of urinary steroid excretion in wild chimpanzees. American Journal of Primatology. 660: 161-166.

Munro, C.J. and Stabenfeldt, G.H. 1984. Development of a microtitre plate enzyme immunoassay for the determination of progesterone. Journal of Endocrinology. 101:41-49.

Nash, I.T., Fritz, J. Alford, P.A., and Brent, L. 1999. Variables influencing the origins of diverse abnormal behaviors in a large sample of captive chimpanzees (*Pan troglodytes*). American Journal of Primatology. 48: 15-29.

Parnell, R.J. 2002. Group size and structure in western lowland gorillas (*Gorilla gorilla gorilla*) at Mbei Bai, Republic of Congo. American Journal of Primatology. 56: 193-206.

Perloe, S.I. 1986. Conflict affiliation and mating, and the effects of spatial confinement in a captive group of squirrel monkeys (*Saimiri sciureus*). In: *Current Perspectives in Primate Social Dynamics*. D. M. Taub and F. A. King (eds.). New York: Van Nostrand Reinhold, 89-98.

Pedersen, V. 1996. Combined behavioural and physiological measurements as a basis of the assessment of animal welfare. Acta Agriculturae Scandinavica Supplement, 27: 46-49.

Remis, M.J. 1997. Western lowland gorillas (*Gorilla gorilla gorilla*) as seasonal frugivores: use of variable resources. American Journal of Primatology. 43: 87-109.

Robbins, M.M. 1999. Male mating patterns in wild multimale mountain gorilla groups. Animal Behaviour. 57: 1013-1020.

Robbins, M., Bermejo, M., Cipolletta, C., Magliocca, F., Parnell, R.J., and Stokes, E. 2004. Social structure and life-history patterns in western gorillas (*Gorilla gorilla gorilla*). American Journal of Primatology. 64: 145-159.

Rushin, J. 1991. Problems associated with the interpretation of physiological data in the assessment of animal welfare. Applied Journal of Behavioral Science. 28: 381-386.

Rushen, J., 2000. Some issues in the interpretation of behavioral responses to stress. In: *The Biology of Animal Stress*. Moberg, G.P. and Mench, J.A (eds). New York: CABI Publishing, 23-42.

Sandøe, P. & Simonsen, H.P. 1992. Assessing animal welfare: where does science end and philosophy begin? Animal Welfare. 1: 257-267.

Sannen, A., Van Elsacker, L., and Eens, M. 2004. Effect of spatial crowding on aggressive behavior in a bonobo colony. Zoo biology. 23: 383-395.

Schaller, G. 1963. *The Mountain Gorilla: Ecology and Behavior*. University of Chicago Press, Chicago.

Schmid, J., Heistermann, M., Gansloßer, U., and Hodges, J.K. 2001. Introduction of foreign female Asian elephants (*Elephas maximus*) into an existing group: Behavioral reactions and changes in cortisol levels. Animal Welfare. 10: 357-372.

Schnell, C.R. and Wood, J.M. 1993. Measurement of blood pressure, heart rate, body temperature, ECG, and activity by telemetry in conscious unrestrained marmosets. Fifth FELASA Symposium: Welfare and animals. 107-111.

Shepherdson, D.J., Carlstead, K.C., and Wielebnowski, N. 2004. Cross-institutional assessment of stress response in zoo animals using longitudinal monitoring of faecal corticoids and behavior. Animal Welfare. 13: S103-113.

Smith, R.F. and Dobson, H. 2002. Hormonal interactions within the hypothalamus and pituitary with respect to stress and reproduction in sheep. Domestic Animal Endocrinology. 23: 75-85.

Stoinski, T.S., Czekala, N., Lukas, K.E., and Maple, T.L. 2002. Urinary androgen and corticoid levels in captive, male western lowland gorillas (*Gorilla g. gorilla*): age- and social group-related differences. American Journal of Primatology. 56: 73-87.

Stokes, E.J. 2004. Within-group social relationships among females and adult males in wild western lowland gorillas (*Gorilla gorilla gorilla*). American Journal of Primatology. 64: 233-246

Stokes, E.J., and Parnell, R.J. 2003. Female dispersal and reproductive success in wild western lowland gorillas (*Gorilla gorilla gorilla*). Behavioral Ecology and Sociobiology. 54: 329-339.

Taussky, H.H. 1954. A microcolorimetric determination of creatinine in urine by the Jaffe reaction. Journal of Biological Chemistry. 208: 854-861.

Thomas, P.R. and McCann, C.M. 1997. The effectiveness of cortisol as a physiological marker of well-being in zoo animals. 1997 AZA Annual Conference Proceedings. 524-529.

Tutin, C.E.G. 1996. Ranging and social structure of lowland gorillas in the Lopé Reserve, Gabon. In: *Great ape societies*. McGrew, W.C., Marchant, L.F., and Nishida, T. (eds). Cambridge University Press; Cambridge, 58-70.

Van Eekelen, A.P.J., Kerkhof, G.A., and van Amsterdam, J.G.C. 2003. Circadian variation in cortisol reactivity to an acute stressor. Chronobiology International. 20: 863-878.

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

Weiss, J.M., Sundar, S.K., and Becker, K.J. 1989. Stress-induced immunosuppression and immunoenhancement; cellular immune changes and mechanisms. In: *Neuroimmune Networks; Physiology and Diseases*. Goetzl, E.J. and Spector, N.H. (eds). Wiley-Liss: New York, 193-206.

Wielebnowski, N.C. 1999. Behavioral differences as predictors of breeding status in captive cheetahs. Zoo Biology. 18: 335-349.

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

Wielebnowski, N. 2003. Stress and distress: evaluating their impact for well-being of zoo animals. Journal of the American Veterinary Medical Association. 223: 973-976.

Woods, S. 2001. Stress-related ear covering by captive great apes: a second look. In: *The apes: challenges for the 21st century, conference proceedings.* Chicago Zoological Society; Chicago, 94-95.

Yamagiwa, J. 1986. Activity rhythm and the ranging of a solitary male mountain gorilla (*Gorilla gorilla beringei*). Primates, 27: 273-282.

Yamagiwa, J. 1987. Intra- and inter-group interactions of an all-male group of Virunga mountain gorillas (*Gorilla gorilla beringei*). Primate*s*, 28: 1-30.

Zar, 1999. Biostatistical Analysis. 4th ed. Prentice-Hall, Inc. Upper Saddle River, New Jersey, 663 pp.

Ziegler, T.E., Scheffler, G., and Snowden, C.T. 1995. The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, *Saguinus oedipus.* Hormones and Behavior. 29: 407-424.