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The Relationship Between Salivary Cortisol Levels and Stressful Behaviors upon the Introduction of a New Exhibit Mate in Captive Black and Gold Howler Monkeys (*Alouatta caraya*) at the Hattiesburg Zoo

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by

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ABSTRACT

THE RELATIONSHIP BETWEEN SALIVARY CORTISOL LEVELS AND STRESSFUL BEHAVIORS UPON THE INTRODUCTION OF A NEW EXHIBIT MATE IN CAPTIVE BLACK AND GOLD HOWLER MONKEYS (*ALOUATTA CARAYA*) AT THE HATTIESBURG ZOO

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Salivary cortisol levels were taken on a male and a female howler monkey and compared with behavioral observations during their introduction to one another into an exhibit at the Hattiesburg Zoo in order to determine the link between behavior and stress. This study sought to answer the following research questions: What behavioral responses occur when two howler monkeys are introduced into the same exhibit at a zoo? How stressed are the animals at different stages of the introduction? And, what is the correlation between behaviors and hormones, if any?

The study spanned four phases including a baseline phase, the initial introduction phase, a post-introduction phase after the monkeys were allowed access to each other at night, a secondary introduction phase where four Macaw parrots were introduced into the exhibit, and a return to the third phase when the birds were removed. Behavior observations and salivary cortisol levels were also analyzed with regards to the four phases of the introduction.

Introduction Phase C, overnight access, was determined to an impact on several of the behaviors that were monitored. Cortisol levels for Monkey 1, the resident howler,

increased on day one of the introduction and returned to baseline range shortly after. Cortisol levels for Monkey 2, the introduced howler, increased after the birds were introduced into the exhibit. No strong relationship was found between behavior and cortisol levels for Monkey 1. Pearson's correlations indicate a relationship exists between increased salivary cortisol levels and howling and biting for Monkey 2. These results suggest that the behaviors of howling and biting could be behavioral expressions of physiological stress. Therefore it is important for zoos to monitor this behavior in case an intervention is needed.

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CHAPTER I

INTRODUCTION

The study of non-human primates has long been a part of anthropology due to their close evolutionary relationship with humans. Primates display many behavioral responses to environmental pressures; assuming that these responses are by-products of natural selection, understanding the relationship between non-human primates and the environment can aid anthropology by providing insight into the behavioral and environmental factors that have shaped the evolution of humanity. Traditionally primates have been observed in their natural habitat; however more and more studies are being conducted on captive animals due to their availability and accessibility. The behavior and physiology of captive populations are often compared with free-ranging populations in order to determine the state of their wellbeing. Many zoo studies are meant to delineate the health and general state of the resident population. Zoos provide an excellent locality for the observation of captive animals because they are controlled environments. This type of environment allows for not only behavioral studies, but also provides human access to the animals, which makes possible the collection of bodily fluids that can measure physiological changes associated with behavior and environmental changes.

One bodily fluid often collected is saliva which can be used to monitor cortisol levels. Cortisol is a hormone that is released as a response to stress, and therefore its levels can be compared to behavioral observations as a means to link responsive behaviors to physiological stress. Stress literature indicates that exposure to psychological stressors for short periods of time can increase the levels of cortisol in saliva as well as blood and urine (Kemeny 2003). This type of research has implications for anthropology because it will help better shape our understanding of the stress response in primates as a whole; for zoos because it will help to understand what happens physiologically with their animals; and for behavioral neuroscience because it will aid in the understanding of the relationship between physiology and behavior.

The literal meaning of the term stress has been the source of much debate in the academic bubble that is stress literature. Often times the term is used to encompass the entire myriad that is the stress response, or stress can be used to describe one aspect of the response. In everyday conversation, it is usually used to describe a state that we find ourselves in, or could perceive ourselves being in the future, or a state we can recall being in the past. For most humans, day to day stressors incite worry, they are the things we fret about; stressors cause us to miss sleep, lash out at loved ones, abuse substances, give up, or even try harder. When we are stressed our heart rate increases, we have tension headaches, we can have heart attacks, anxiety attacks, memory loss and a number of other maladies. In fact, the longer we stay stressed, the greater the possibility of doing significant harm to our bodies (Sapolsky 1994). The stress response is essentially the same for all mammals, but the things that actually trigger it are unique to the individual, based on genetics, environmental influences, and personality. We all respond uniquely to the situations that life brings about.

The purpose of the study was to determine the relationship between outward signs of behavioral stress and the internal physiological response to stress that is undertaken by two howler monkeys, a male and a female black and gold howler monkey (*Alouatta caraya),* upon their introduction to one another in a captive environment. This study sought to answer the following questions: What behavioral responses occur when two howler monkeys are introduced into the same exhibit at a zoo? How stressed are the animals at different stages of the introduction? And, what is the correlation, if any, between behaviors and hormones?

I monitored the behavior of each animal five days per week over a five week period using the focal animal method described by Altman (1973). Saliva samples were collected in the morning and afternoon on days when behavior was monitored. There was no basis for comparison of the levels of the two howlers because they are of different sexes. Therefore each individual's behavior can only be compared to their own cortisol level. The control for this study was behavioral observations and cortisol levels obtained for each individual for one week prior to their introduction. I hypothesized that (1) the frequency of stressful behaviors will increase immediately after the introduction and will fall towards the end of the study; (2) salivary cortisol levels will increase immediately following the introduction and will fall towards the end of the study; and (3) there will be evidence of a correlation between stressful behaviors and higher levels of salivary cortisol.

This study was designed to give both zoo workers and researchers a better understanding of the behavior of captive howlers. In addition to the behavioral data that was recorded, baseline salivary cortisol levels were obtained for *Alouatta caraya* that could be valuable for future research. The comparison of the behavioral and physiological data can help us identify behaviors that are triggered by, or related to, the stress response.

CHAPTER II

LITERATURE REVIEW

Howler monkey behavior and stress are the main subject matter related to this project. The literature relevant includes that which characterizes and describes the nature of black and gold howler monkeys, explains stress and the stress response, and traces the history of primate stress investigations. This review provides an overview of howler monkeys, and investigates prior research in both captive and free-ranging populations. It also considers the stress response and the role of cortisol and the use of cortisol levels as a means of monitoring stress in non-human primates.

Howler Monkeys

The study of primates in anthropology has generally focused on Old World primates. If the purpose of studying primates, in a field in which humans are the focus, is to gain insights into our evolutionary history, then investigating the non-human primates who are most closely related to humans evolutionarily is valid. However, by primarily investigating solely the Haplorrhini and Catarrhini infraorders, we miss a large portion of the big picture. New World monkeys can be valuable to anthropology for their similarities to Old World monkeys, but can perhaps be even more valuable for their differences. Old and New World monkeys have undergone similar adaptations without gene flow between the groups, a phenomenon known as parallel evolution. By understanding the ecology, foraging strategy and adaptations of New World monkeys, we can better understand the reasons behind how early hominids and modern humans evolved the unique characteristics that they possess (Kinsey 1986).

The Platyrrhini are the rogue monkeys in anthropology because they evolved independently from the Catarrhini in an isolated region, South America, which was separated from Africa by the Atlantic Ocean. The two infraorders are believed to have diverged in the early Oligocene (possibly as early as the Eocene epoch), roughly 34 million years before present (Fleagle and Kay 1997). The earliest known Platyrrhine fossils were found in Bolivia, and came from the stratigraphic level indicating they are from the late Oligocene (Fleagle and Kay 1997).

New World monkeys differ slightly from Old World monkeys in that they possess 36 teeth rather than 32, having an extra premolar. While both Old and New World monkeys have dry noses, those of the New World are flat where as the nostrils of Old World monkeys point downward. In general, the Platyrrhines have a smaller body size than Catarrhines, and many, including howler monkeys, possess a grasping prehensile tail that is not seen in the Old World (Redmond 2008).

One interesting by-product of the parallel evolution of Old and New World monkeys is the degree of color vision they have. Old World monkeys and humans are trichromatic, meaning that our eyes have three different cones for processing color. Most New World monkeys are dichromatic, meaning that they cannot distinguish hues as distinctly as Catarrhines can. However, some New World females are trichromatic with heterozygous alleles for the color vision gene; females that are homozygous for the gene and males that are either heterozygous or homozygous are dichromatic (Herlyn and Zischler 2006). The only New World monkeys that are traditionally trichromatic like their Old World relatives are found in the genus *Alouatta*.

Howler monkeys make up the genus *Alouatta.* Nine species of howler monkeys exist in the wild, and they range from Central America to the majority of the South American continent. This study will involve one species, *Alouatta caraya*, or the black and gold howler. Black and gold howlers are found in Argentina, Brazil and Paraguay. They are foliovore-frugivores with their diet consisting equally of fruits, leaves, flowers and buds (Kinzey 1997). Howlers live a sedentary lifestyle with up to 80 percent of their day spent resting in the trees (Redmond 2008). This is a necessary adaptation because a large portion of the diet consists of leaves, which are low in energy. They are arboreal quadrupeds moving through the tree tops on all fours using their prehensile tail as an anchor while resting on branches (Kinzey 1997). Particularly advantageous to this study is that howler monkeys have adapted large salivary glands that produce a great supply of saliva that is necessary to break down the poisonous tannins found in leaves (Redmond 2008).

Black and gold howler monkeys were once believed to be two distinct species, a misconception perpetuated by their extreme sexual dimorphism in color or sexual dichromatism. The adult males of this species have a black coat color while the females are golden or blond. Young males are born golden and begin to turn dark upon maturity. They are also sexually dimorphic in size, with males weighing roughly twice as much as the females. Females generally do not exceed 6.5 kg while males rarely exceed 9.5 kg (Pope 1966).

These monkeys occupy wooded areas and prefer to feast on fresh leaves. Their average group size is between six and nine individuals; however they have been observed living in pairs or large troops of up to 20 (Rumiz 1990). It is most likely that those observed residing in pairs are adolescents that have migrated from their parental group, which is frequently done just prior to maturity (Rumiz 1990). During this time they may even live alone, which is common before forming or joining an adult group.

An adaptation that is unique to howler monkeys and particularly *Alouatta caraya* is an enlarged hyoid. The bone sits in-between the chin and the thyroid gland and provides humans the physical logistics that are necessary for speech. In howler monkeys it is extremely large and oscillates while howling, acting as a vocal amplifier (Kinzey 1997). Black and gold howlers are the loudest animals in the world, their territory roars can be heard from miles away (Kinzey 1997). The male hyoid is much larger than that of the female, and as result males are much louder and roar more often.

Howlers have two types of calls. One is the roar, usually heard upon waking in the morning, and the other is a sort of screechy grunt, often referred to as a bark that is continuous with different sounds being expelled upon inhalation and exhalation (de Cuhna 2006). In a way it sounds like an amplified version of a bullfrog's call. Females usually join in with the male vocalizations which can strengthen the pair bond between mated individuals (Kinzey 1997). In free ranging animals group choruses of long calls, or the roar, are usually heard in the mornings and are thought to be signals to solitary individuals and other groups indicating territory, group size, and the number of males and females within the group (Kinzey 1997).

Behavioral Studies in Free Ranging Populations

Among all the New World monkeys, members of the genus *Alouatta* have been the subject of field studies the most often and for the longest time period, going back to the 1920s. A typical day in the life of a black and gold howler monkey begins with providing a wake-up call to the forest. The Alpha male leads his group with long calls at dawn nearly every day with subordinate males and females joining in as a chorus (da Cunha 2006). These daily calls announce the territory of the group, and because they occur generally as a whole group chorus, they signal other groups of their composition with regard to sex. Following the morning roar session, group members leave their nighttime sleeping tree and travel to a different location in their range to forage for leaves and fruits (Young 1982). The remainder of the day follows a similar pattern; after the morning meal the group may move to leafless trees to rest in the sun. The majority of the day is spent resting, and the monkeys are likely to repeat this foraging and resting pattern midday and in the afternoon before returning to their sleeping tree.

There has been much speculation about the reasons behind choice of location and posture during the resting period of howlers. Young (1982) has suggested that thermoregulation allows howlers to direct energy that might be consumed maintaining body temperature to the digestive tract, aiding in the process of fermenting the tough and low caloric value leaves that make up such a large part of their diet. Thermoregulation has also been hypothesized as a possible source for the sexual dichromatism of *Alouatta caraya* (Bicca-Marques 1998). However, after further investigation there were no differences between the sexes found with regard to thermoregulation. Therefore, sexual dichromatism is most likely the result of mate selection by females, not a difference in the way males and females process heat (Crockett 1998).

Young (1982) documented a confrontation between a group of howler monkeys and turkey vultures on Barro Colorado Island in Panama in March of 1974. The howler group came upon a tree, barren of leaves, that was occupied by seven turkey vultures.

The adult males began roaring loudly, shortly followed by the females and juveniles joining in; the males would advance toward the vultures roaring loudly with very stiff and alert body posture. However the vultures did not move but became vocal themselves and flapped their wings (Young 1982). The howlers eventually retreated and did not return until 30 minutes after the vultures left.

Young (1982) concluded that the encounter was based on competition for a valuable resource; the exposure of the branches to sunlight. Once the tree was theirs for the taking, they assumed normal sunning positions resting on branches most often with their backs facing the sky but occasionally lying on their backs with their chest exposed (Young 1982). A study conducted by Bicca-Marques (1998) on black and gold howlers at Estância Casa Branca, Alegrete-RS, Brazil, confirmed the relationship between energy conservation and posture based on temperature. In cold weather the monkeys were observed curled up with their backs exposed to the sky; in warmer weather they were most likely lying with their limbs spread out allowing heat to dissipate from their body. These patterns were consistent through both sexes and age range further dispelling the hypothesis that thermoregulation is the source of sexual dichromatism in *Alouatta caraya* (Bicca-Marques 1998).

Allogrooming is a common occurrence for black and gold howlers. Grooming sessions are generally rather short in duration, and most often adult females groom males and juveniles (Kinzey 1997). Acts of aggression are not common for the genus *Alouatta*. When they have been documented, such acts normally occur between males and are often associated with leaving a group or attempting to join one (Kinzey 1997).

Behavioral Observations in Captive Populations

Jones (1983) conducted a study on captive black howler monkeys defining for the first time their social behavior. She focused on social competition and the behavioral frequency of damaging and non-damaging behaviors, finding that the occurrence of less aggressive behaviors escalating to more aggressive behaviors happened only rarely. This relationship is impacted by ritualized behaviors that provide a signal that prevents outward aggression. Howler monkeys are not known for acts of aggression due to their biological need to conserve energy. Aggressive displays such as fighting are known as damaging behaviors while non-aggressive acts such as grooming are called non-damaging behaviors (Fagen 1980). Both types of behavior can lead to energy expenditure but damaging behaviors more so than non-damaging ones. Howler monkeys normally would live in a group dynamic consisting of several females and a few males up to a total of 20 individuals. In a large group dynamic, damaging behaviors are those that incur reproductive cost brought about by fighting (Jones 1983).

Non-damaging behaviors have been studied in howlers in the past because they are generally more passive than other polygamous monkeys (Jones 1983). Non-damaging behaviors are associated with a noncompetitive social organization (Jones 1983). This behavior set includes allogrooming and ritualized behaviors such as clitoral and vulval display that can serve as signals to avert aggressive behaviors. For example, a female can participate in a vulval display, showing her backside to an advancing male, signaling him to back off or leave her alone. Many non-damaging behaviors are known as tactile behaviors which can be categorized as either ritualized or stereotyped (Maynard Smith and Price 1973). Tactile behaviors are those that involve touch such as allogrooming. Ritualized behaviors include such things as genital displays and greeting ceremonies and are associated with conflict resolution and decrease the likelihood of damaging or fighting behavior. Stereotyped behaviors such as play, grooming, supplantation and copulation generally occur more frequently (Jones 1983).

The Stress Response

As touched on earlier, there is no singular definition of the term stress. In the biology and physiology of animals, researchers focus on the stressor and the stress response. To define stress in terms of a stressor is to say that a stressor triggers stress or the loss of physiological homeostasis (Reeder 2005). Stressors are the outside forces that throw off the internal hormonal balance of the body. Sapolsky (1993) defines stressors as anything that disrupts physiological balance. Stressors can affect an animal physically, such as injuries incurred during battle, or psychologically, such as fear of an event or undesirable outcome, or it can be a combination of the two. When stressors induce the disruption of physiological balance, the body invokes the stress-response to restore this imbalance. The stress response is an adaptation designed to correct such stressor induced imbalances (Sapolsky 1993). Through all of this we can argue that a workable definition for physiological stress can be both the occurrence of a stressor, the disruption, as well as the body's attempt to return to homeostasis, or the stress response (Kemeny 2003). For an organism to be stressed, first they must suffer the occurrence of a stressor, which then skews the hormonal balance within the body, which then triggers the stress response to restore the balance.

This all sounds fairly simple; however the stress response is a brilliant and complex mechanism that surprisingly is essentially the same for all mammals. The major difference that I can see from organism to organism is not in the actual physiological mechanism of the stress response, but in what constitutes a stressor, or what causes the stress response to be invoked. So let us explore the multifaceted cascade that is the stress response.

The stress response is tied to two endocrine systems involving the adrenal gland: the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis (Reeder 2005). The first involves the secretion of epinephrine, and the HPA axis involves the secretion of glucocorticoid hormones such as cortisol. Physiological alterations to these two systems are believed to invoke behaviors that will help an animal deal with any threat they may be experiencing (Kemeny 2003).

The secretion of epinephrine is associated with the fight or flight response or the rush of adrenaline felt in response to a threatening situation. This rush of adrenaline or epinephrine occurs instantaneously after the occurrence of a stressor (Reeder 2005). Fight or flight surely is something most humans can relate to. Happening upon a snake in the woods would be sufficient enough to invoke the fight or flight response. Natural reactions could include running away screaming or finding a weapon to kill the snake. Imagine stepping on the snake instead of just seeing it, a reasonable reaction would be to scream and simultaneously jump off of it and then run away praying you were not struck. The notion that the snake did strike and you could not feel the pain as you were fleeing is a byproduct of the release of epinephrine, blocking the pain signals to the brain which allows you to escape or attempt to kill the snake as opposed to writhing in pain on the forest floor from a deadly snake bite.

The sympathetic nervous system is one portion of the autonomic nervous system that also includes the parasympathetic nervous system. The parasympathetic nervous system regulates involuntary bodily functions such as breathing and control of heart rate. Aspects of this system react in response to the activation of the sympathetic nervous system invoked by a stressor (Kemeny 2003). Epinephrine is released into the bloodstream by pathways of the sympathetic nervous system immediately after the occurrence of a stressor; almost simultaneously the parasympathetic nervous system responds by increasing the heart rate and other involuntary functions such as breathing.

Therefore, you see the snake and your body reacts. Firstly, fibers within the sympathetic nervous system release the neurotransmitter norepinephrine which in turn signals the adrenal medulla portion of the adrenal glands to release the epinephrine into the bloodstream (Kemeny 2003). Due to this chemical flood of epinephrine in your bloodstream, your heart begins beating rapidly, you begin to breathe harder and faster, and blood rushes into your muscles which would either enable you to run away in a hurry or fight if you must. The fight or flight response is a key adaptation and is essential to keep mammals alive, unfortunately for us humans and non-human primates, it does not always occur as a result of life or death situations.

The next step in the cascade involves the HPA axis. For the sake of the story, let us say that the snake was not poisonous, it did not bite you and you got away. Within a minute, you have put a couple of football fields in between you and the last known location of the snake. What does your body do now? Its homeostatic balance is thrown off due to the surges of norepinephrine and epinephrine in your bloodstream, so now what happens? Glucocorticoid hormones are released into the bloodstream.

Upon the perception of a stressor by the brain, neural pathways signal the hypothalamus to release corticotropin-releasing hormone, or CRH. At the base of the hypothalamus is a tiny gland known as the pituitary gland that responds to the CRH by releasing adrenocorticotropic hormone, or ACTH. Located just above the kidneys are the adrenal glands. The adrenal glands are divided into two distinct sections. The interior of the gland consists of the adrenal medulla which is responsible for the release of epinephrine. The exterior of the gland is made up of the adrenal cortex which responds to the release of ACTH by releasing glucocorticoids. In humans and non-human primates the dominant glucocorticoid hormone released is cortisol (Sapolsky 1993). Epinephrine is released seconds after a stressor occurrence, while it takes cortisol minutes to reach the bloodstream. Cortisol levels are at their highest 20 to 40 minutes after the invocation of the stress-response (Kemeny 2003). The entire stress-response takes about an hour to restore physiological balance after the onset of a stressor.

Seeing as you are still alive and are now far away from the snake, we know that the flood of epinephrine into your bloodstream was crucial to your survival, the increased blood flow to your muscles allowed you to run away which begs the question: What good is having all that cortisol circulating through your body? What does the cortisol do? Put simply it acts to restore homeostasis, but before it does that, it sort of helps the epinephrine act. Once glucocorticoids are released, they signal the liver to mobilize stores of fat and protein and alter their composition into a simpler sugar, glucose (Matteri et al. 2000). The glucose then travels through the body providing muscles, organs and tissues with energy thereby sustaining the initial effects provided by the rush of epinephrine at the onset of the stressor.

Cortisol acts as both a key physiological component necessary for dealing with a stressor and also enables the body to shut off the stress response once it is no longer needed. It shuts down the stress response by what is known as negative feedback inhibition, by shutting off the response of the HPA axis (Matteri et al. 2000). As was mentioned before, the entire cascade that leads to the release of cortisol from the adrenal cortex begins with the release of CRH from the hypothalamus. Essentially there is a set limit of cortisol concentration that the brain will allow. If the level is below this limit, the brain continues to secrete CRH leading to the release of more cortisol; if the level has surpassed the limit the brain is signaled to stop secreting CRH, which will ultimately result in a cease fire of the stress response (Sapolsky 1994).

Primate Studies and Cortisol

The study of primates and their response to stress has a long history. The true pioneer in this field is Robert Sapolsky who began investigating primate stress in a group of free-ranging olive baboons in 1978. He was particularly interested in the influence behavior had on stress related diseases and vice versa (Sapolsky 2001). Since this time period, research on the topic has actually become quite common. The appeal is plain to see; modern human society is filled with complex psychological stressors that are ever-changing and often compounding. Non-human primates are similar to us physiologically, their bodies respond to stress in the same manner ours do. The role of scientific experimentation has always been to minimize variables and to attempt to understand phenomena by utilizing a controlled environment. Since modern human society is so complex, there are too many possible sources of stress to examine all of them. This is why primate studies in stress are so appealing. Primates are intelligent creatures that often live in their own version of societies, and while their sources of stress are by no means static, they do not change as frequently as ours and they certainly do not compound the way ours do. By studying non-human primates we get a glimpse of a simpler version of stress and life.

When Sapolsky began looking at stress hormones in his baboons, he had to obtain a blood sample to retrieve cortisol levels. This was not an easy task and there were a lot of limitations caused by it. In order to get a blood sample from one of the baboons he had to shoot them with a tranquilizer dart, stick them with a needle and take their blood (Sapolsky 1990). This was the only method available to retrieve cortisol levels at the time. What wound up happening because of this is that the method dictated what could be studied. Sapolsky could not shoot female baboons with tranquilizer darts because generally they were always pregnant or nursing, and so he could only study male baboons (Sapolsky 2001).

In the early 1990s a new method for retrieval of cortisol levels began to appear in stress studies. Urine and feces were determined to be suitable conduits for cortisol. These bodily fluids were first determined to be reliable measures of cortisol in a study conducted in Rocky Mountain bighorn sheep (Miller et al. 1991). Subsequently fecal cortisol studies have become more prevalent. It is a particularly useful measure when studying stress in free-ranging populations due to its procurement's non-invasive nature. Fecal cortisol is also being utilized to study stress in captive animals for the same reasons. The trouble with feces is that there are many factors not related to stress that can alter the excretion of fecal glucocorticoids and lead to difficulty in drawing conclusions from data (von der Ohe and Servheen 2002). Such factors include diet, adaptation, reproductive events, and sex among others.

Another problem with fecal cortisol is that it is not an immediate measure of a stressor. Researchers cannot instigate a stress event and immediately get a fecal cortisol level. They have to wait for the animal to defecate, and even if samples are retrieved at regular intervals, there is no way of ruling out other stressors. That is why there are a lot of fecal studies that compare different groups of the same species. An investigation was conducted on two different groups of black howler monkeys (*Alouatta pigra)* to determine if the condition of their habitats influenced the physiological stress response. The researchers determined that the monkeys living in fragmented forest had higher fecal cortisol metabolites than their counterparts that lived in continuous forests (Martinez-Mota et al. 2007). In such a case, fecal cortisol is obviously the best method for retrieval.

Kirschbaum and Hellhammer (1989) deemed salivary cortisol a valuable measure of HPA axis activity for clinical and scientific research. In primate literature salivary cortisol research really did not become common until the 21st century. Currently the use of salivary cortisol is a popular new way of measuring stress in captive primates. The benefits of this method are that it is not as invasive as obtaining cortisol from serum and it allows researchers the ability to measure the stress response quickly after the onset of a stressor. Cortisol passes into saliva by passive diffusion; hormones in the blood enter into the mouth through capillary walls that surround saliva glands (Salimetrics, The Entry of Substances into Saliva and the Effect of Flow Rate on Their Concentrations 2008). Cortisol concentrations are much lower in saliva than they are in blood due to proteins called corticosteroid binding globulin that attract cortisol in the blood; these molecules are too large to pass through the capillaries in the mouth (Salimetrics, The Entry of Substances into Saliva and the Effect of Flow Rate on Their Concentrations 2008). This leaves only one to ten percent of cortisol molecules that are unbound to a protein and are small enough to pass into saliva. For these reasons, any amount of blood present in the mouth can skew salivary cortisol data.

Salivary Cortisol and Primate Studies. Salivary cortisol has been utilized in primate studies rather often over the last decade (Lutz et al. 2000). Unfortunately none of these studies have been conducted on black and gold howler monkeys. The first methods developed for collecting saliva samples from unrestrained adult monkeys were tested using a group of male rhesus monkeys. These monkeys were trained according to the screen method, which involves licking gauze covered with screen, or to chew or suck on rope attached to a pole (Lutz et al. 2000). Both methods were deemed viable, but some monkeys were prone to bite off the rope. Some researchers have opted against using the rope for this very reason, with concerns of it potentially being a choking hazard, coupled with possible sample loss. No significant difference was found in the cortisol levels obtained from either method. For the rhesus monkey, the mean cortisol level was 0.84 ug/dL and ranged from 0.27-1.77ug/dL (Lutz et al. 2000).

Squirrel monkeys are a New World group that has often been the subject of salivary cortisol studies. They are very small monkeys, generally weighing 1-2 pounds, which are known to have high salivary cortisol levels. One study utilized the “rope” technique designed for rhesus monkeys where dental rope was attached to a pole. This study found the salivary cortisol levels in squirrel monkeys to follow the general trend found in all primates in which cortisol is high in the mornings and gradually declines over the course of the day (Tiefenbacher et al. 2003). Morning salivary cortisol levels in squirrel monkeys were found to range between 17.1-37.9 ug/dL (Tiefenbacher et al. 2003).

Another study in squirrel monkeys tested salivary cortisol levels prior to an experimental condition and afterwards (Fuchs et al. 1997). This study involved two squirrel monkeys, one male and one female. Saliva samples were collected in this study using a cotton swab. The non-experimental portion involved the animals sitting in a primate chair where they were restrained. The monkeys also were restrained in the chair during the experimental phase and had to complete a task that involved pushing a stick, and the female participated in a second experimental phase where she had to determine the difference between noise, a tone and a twitter-call (Fuchs et al. 1997). The female exhibited little difference in cortisol concentrations for the differing sessions, while the male showed significant increases for them.

The most significant study in relation to the howler study involves fecal and salivary cortisol concentrations in woolly and spider monkeys from various zoos in the United States and Europe. This study is important because woolly and spider monkeys are both New World monkeys and their size and weight is analogous to black and gold howlers. This investigation focused on the differences in diet composition between the zoos and the effects it had on cortisol concentrations in both feces and saliva. Saliva samples for this study were collected using dental rope on spider monkeys from four zoos where a total of 66 samples were analyzed (Ange-van Heugten et al. 2009). Salivary cortisol levels were lowest in zoos that had the most nutritious diet for primates. For the zoo that had the best primate diet spider monkey salivary cortisol ranged from 2 ± 3.2

ug/dL and the zoo with the poorest diet had salivary cortisol levels of 17 ±3.7 ug/dL (Ange-van Heugten et al. 2009). Woolly monkeys did not contribute saliva samples.

From the studies mentioned above we know that salivary cortisol is a good measure of HPA axis activity. We also know that in general terms New World monkeys have higher cortisol concentrations in the saliva than do Old World monkeys. Regrettably there are no studies that provide salivary cortisol levels in howler monkeys for comparison with the results obtained in this study. However, based on the study conducted on spider monkeys at various zoos, we have an idea of how salivary cortisol ranges in a New World monkey that is similar in size and weight to black and gold howlers.

A study very similar to this howler study was conducted on three male western lowland gorillas (*Gorilla g. gorilla)* in which salivary cortisol levels were compared with social behavior and activity. Ironically, this study was conducted at the Cleveland Metroparks Zoo, the same place from which the Hattiesburg Zoo acquired the male howler (Monkey 2). Saliva samples were obtained using dental rope that the gorillas were trained to chew and then trade through a fence in exchange for food (Kuhar et al. 2005). Behaviors were monitored using a combination of two sampling methods. The behaviors that were measured against cortisol were Affiliative, which involved touching, grooming, and play; Agonistic, which included aggressive postures and behaviors; Inactive, in which the animals were idle; and Neighbor where the focal animal was within three feet of another animal (Kuhar et al. 2005). Reported salivary cortisol levels ranged between 0.05 ug/dL and 0.35 ug/dL; this study did not find a link between observed behaviors and salivary cortisol levels (Kuhar et al. 2005).

Non-human primates are popular subjects for stress research due to their physiological similarities to humans and their social nature. No prior study has shown evidence linking a specific type of behavior with high cortisol. The howler study has the potential to be influential in this area because of the method invoked. Previous investigations have attempted to align categories of behavior with stress; this study makes record of the frequency of specific behaviors that fall into a number of categories. This allows for the relationship to be seen between individual behaviors and daily salivary cortisol levels.

CHAPTER III

METHODS AND MATERIALS

This chapter will focus on the overall design of this project. Materials included the monkeys, cortisol sampling supplies, and the enclosure. The method utilized for this study involved a single-case design, focal animal sampling, and a clicker/reward method for collection of salivary cortisol.

Research Design

The opportunity for this study transpired when the Hattiesburg Zoo learned they would acquire a male howler from the Cleveland Zoo. John Wright, the former general curator at the zoo, was interested in a project that monitored the introduction of the two monkeys, and he suggested the use of salivary cortisol as a measure of stress. Thus, my study is essentially a single-subject design with two participants. Single-subject designs fall within the category of quasi-experimental research which generally involves one or more subjects that are not randomly assigned to an experimental group, an independent variable, and a control period followed by one or more periods of intervention (Gliner et al. 2009:49).

For this investigation, there were two participants that received three experimental treatments after a baseline period. Each participant was tested on two differing levels, the independent variable was salivary cortisol levels and the dependent variables were the frequency of a set group of observable behaviors. The baseline phase of this study consisted of saliva sampling and behavior monitoring of each individual separately for one week prior to the introduction.

Based on the nature of the introduction, namely the fact that there is no multi-individual group dynamic present, the Zoo decided to introduce the male and female howler by putting them together in a cage for a few hours and then let them out on exhibit together. Therefore, after baseline controls the intervention phase of the study consisted of Treatment 1 which was the first contact between the two monkeys or their initial introduction, Treatment 2 which allowed the animals to have access to one another overnight, and Treatment 3 which involved the introduction of four macaw parrots into the exhibit.

The aforementioned was the initial design of the study; however due to events that occurred after the birds were introduced, there wound up being a fourth treatment that involved the removal of the third treatment. I will cover this development later in the discussion section, but it is worth mentioning here because instead of having an A, B, C, D design, it actually became an A, B, C, D, C design that resulted from the removal of the birds from the exhibit. To reiterate, this study was designed to answer the following research questions: (1) What behavioral responses occur when two howler monkeys are introduced into the same exhibit at a zoo? (2) How stressed are the animals at different stages of the introduction? (3) What, if any, is the correlation between behaviors and hormones?

Materials

Participants

The primary subjects for this study included a nine year old female and a seventeen year old male black and gold howler monkey. Monkey 1 is a female who has lived at the Hattiesburg Zoo for the majority of her life. Prior to her arrival she was hand raised, making her highly trainable and a perfect candidate to submit saliva for the study. Zoo introductions have been rare in the female monkey's life course; however she has had two previous mates and subsequently has been through a few introductions. Her most recent introduction involved the merging of her living space with that of four macaw parrots. The female howler had been alone in her exhibit for close to a year before the zoo decided to try the parrots in her exhibit to bring some companionship or perhaps to stave off loneliness in an otherwise normally social animal.

Monkey 2 is a seventeen year old male monkey who arrived to the Hattiesburg Zoo from Cleveland. He has spent all of his life in captivity at several different zoos. During his early years he was housed in an indoor exhibit where the only sunlight inside was filtered through an ultraviolet protected skylight. As a result the male has developed spinal dysplasia and is not as traditionally mobile as other howler monkeys. His movements are slow and deliberate, yet he gets around quite effectively, relying heavily on his prehensile tail. In Cleveland he was housed with a harem of females and has been described as very protective of them.

Following a thirty day quarantine period, and a control period, the male monkey was placed on exhibit with the female. The macaw parrots she had been living with were removed prior to the introduction. When the Zoo established enough evidence of a peaceful roommate situation between the two monkeys, the birds were brought back in.

The Enclosure

For the baseline phase of the study, Monkey 2 was housed in the lemur holding building following the quarantine period. In the mornings he was held in a 10 cubic foot cage. The lemurs were out on exhibit when observation began. In the afternoons he was held in a slightly larger outdoor holding cage that was sealed off from zoo patrons. The enclosure where the introduction took place and where Monkey 1 had been housed is roughly 50 feet long, 20 feet high, and 15 feet deep. There are a number of branches rigged between chained-linked fences for climbing and resting. There is a large palm tree and various ferns littering the landscape. The overnight housing facility is known as the aviary and consists of three cages that can be closed off to each other or open through a small door that can allow the animals access to all three cages.

Behavioral Materials

Behavioral observations were monitored using the form I designed which can be seen in Appendix A. I chose this list of behaviors based partially on basic behaviors I observed from Monkey 1 prior to Monkey 2's arrival, partially on common sense logic of what might indicate stress, and partially on a list of behaviors observed by Jones (1983) in her study on the social organization of a group of captive howler monkeys. Descriptions of these behaviors can be found in Appendix B.

Cortisol Materials

Salivary cortisol samples were taken from each monkey using two sorbette swabs. This swab is similar in size and shape to a Q-tip; it consists of a triangular shaped absorbent head attached to a plastic shaft. The Sorbette swab is typically used to collect saliva from infants and children under the age of six and is the best device to collect saliva from non-human primates who have a propensity to chew larger collection tools (Salimetrics, Saliva Collection and Handling Advice 2009). Other materials included a conical storage tube for the Sorbette swab, a centrifuge to extract saliva from the swabs and a large onsite freezer for sample storage (Figure 1).

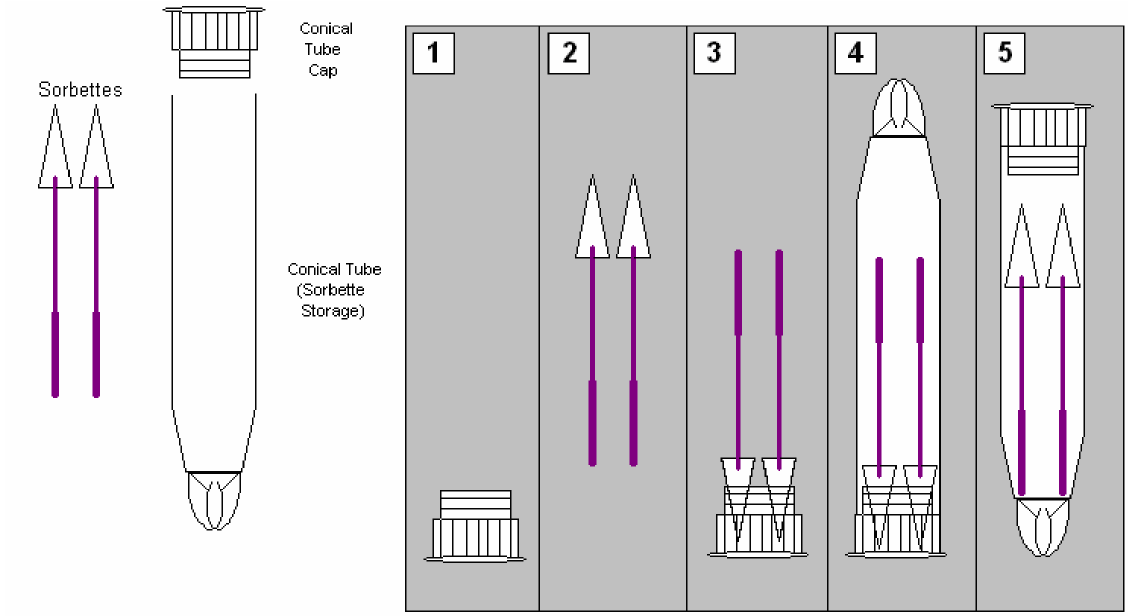


Figure 1. Collection Supplies. 2008 "Saliva Collection Instructions Using the Sorbette." *Salimetrics.* http://salimetrics.com/assets/documents/collection-supplies/, accessed September 23, 2010

Procedure

Behavioral Observation

Focal animal sampling was described by Jeanne Altman (1973) as a means to monitor spontaneous social behavior in a wide array of mammals and other social animals. The method requires a set time period for observations. During this time period, only the behaviors of one animal are to be recorded. Any occurrence of the specified behaviors must be recorded if they occurred during the focal time period. The length of each sample period must be recorded, and the time within that period that the animal is in view must also be noted. The ability to view an animal is more often impeded in studies that take place in the natural environment and should not be a hindrance in a zoo study. Therefore, for the purpose of this study the behaviors of each individual were monitored in five minute increments; during which time they could not be out of view (Altman 1973). This sampling method has been utilized by numerous field studies involving a variety of social mammals and many primates. For example Kuhar et al.'s (2005) gorilla study at the Cleveland Metroparks Zoo utilized the method to monitor affiliative and agnostic behaviors. It is the chosen method for observations in this study because it allows the researcher to quantify behaviors by recording their frequency. This method provides a simple way to connect the prevalence of certain behaviors with daily cortisol levels.

Following Altman (1973), the monkeys were monitored for six hours each day. Each day was divided into a morning and afternoon session. The monkeys were monitored for the occurrence and frequency of behaviors in five-minute increments. Behaviors were sampled randomly with six to nine five-minute focal periods taken per hour. Only one animal was monitored each day. For example, Monkey 1's behavior was monitored on Tuesday, and Monkey 2's behavior was monitored on Wednesday, and so on. Prior to the collection of saliva samples, the monkeys were placed in the same holding cage for 30 minutes in the morning to allow enough time for the stress response to take place. The saliva collection in the afternoon occurred while the animals were on exhibit. During the first week prior to their introduction, the method was altered because the animals were housed separately during this time period. It was necessary to take samples and monitor behavior before their introduction as a control for the study. The control period lasted for six days, during which each animal was monitored for three days with two saliva samples collected each day.

The behaviors that were monitored included stress related behaviors as well as non-stress related behaviors. The non-stress behaviors included grooming, sleep, and degree of movement. The stress behaviors included autogrooming, fear grimacing, body tremors, acts of aggression such as hitting or chasing, and urination, defecation and scent marking. These behaviors were recorded on a standard form and tallied for each monitoring session.

Cortisol Sampling

The monkeys were trained using a clicker/reward method using a Q-tip to collect the saliva. Twenty-five saliva samples were taken from each monkey over a five week period averaging five samples per week. The zoo keeper collected the samples in the mornings 30 minutes after the animals were released together, and at 3:00 pm in the afternoon. Two sorbette swabs were held together in the keeper's hand and then swabbed inside the mouth of each monkey. A single swab must be held in place for 60 seconds to ensure enough saturation for testing to occur. Two swabs were used for each sample to ensure that enough saliva was collected due to the unpredictable nature of the monkeys. The swab could also be reintroduced into the mouth at the same location in 30 second intervals if necessary to ensure saturation (Salimetrics 2009). It was important to try to collect as much saliva as possible; therefore the collection spot was either under the tongue of each monkey or in the saliva rich cheek pouch.

Once the saliva had been collected, the two swabs were placed in a labeled conical storage tube and then centrifuged on site and promptly frozen. The cap of the tube was placed on a flat surface, and the saturated tip of the sorbette swab was inserted into slots in the cap. The conical storage tube was then snapped onto the cap. The tube was then centrifuged for 15 minutes at 3000-3500 rpm to extract the saliva into a receptacle at the bottom of the tube. The tube was then frozen at or below 20°C which is the standard temperature for household freezers. Specimens can remain frozen for two years without affecting testing for hormonal levels (Salimetrics 2009). This process was repeated each time a collection occurred. Once all the samples were collected, they were sent to the Salimetrics Laboratories for analysis. They remained frozen during shipment using dry ice.

Data Analysis

Assuming that primates as a whole exhibit similar responses to stress, the howler study attempted to align increased levels of aggressive behaviors with higher levels of the hormonal indicators of stress or increased salivary cortisol levels. This provided a measure of the impact of various stages of the introduction on behavior and salivary cortisol.

Statistical analysis was used to determine the relationship between the independent variable salivary cortisol levels (X), and the dependent variable behavioral frequency (Y). The frequency of each observed behavior was compared with the mean salivary cortisol levels for the morning session and the afternoon session daily. Salimetrics laboratory ran each sample twice, and the lab report included the mean level obtained from each sample. Morning session cortisol levels were compared to morning session behavioral frequency. Afternoon session cortisol levels were compared to afternoon session behavioral frequency. For the three hour morning session and three hour afternoon session ten five-minute focal periods were chosen randomly for comparison with cortisol levels. Pearson's r, or the correlation coefficient, was calculated to determine the strength of the relationship between salivary cortisol levels and each behavior. SPSS Statistics 17.0 was used to calculate Pearson's r. Morning and afternoon mean salivary cortisol levels (X) were compared against the mean daily frequency observed for each behavior (the average of the frequencies of 10 randomly chosen focal periods = (Y)).

I also looked for general trends about the data by plotting cortisol levels against time to determine at which stages of the introduction cortisol levels change and to determine if the trend is noticeably different from the trend during the control period. This type of comparison also applies to certain behaviors such as autogrooming, determining if there is a trend difference in the occurrence before and after the introduction and at various stages of the introduction.

In summary, the monkeys were monitored on alternate days five days a week for five weeks. The frequency of the occurrence of certain behaviors was recorded and saliva samples were taken twice daily from the focal animal. Salivary cortisol levels were compared with behavioral frequency in order to determine the relationship between each variable. This method allowed for specific behaviors to be compared with salivary cortisol to determine if any correlation exists between the two variables.

CHAPTER IV

RESULTS AND DISCUSSION

The howler study ultimately underwent four phases: A, B, C, and D; and towards the end of the study a return to Phase C (Figure 2). In order to interpret the meanings behind cortisol and behavioral sampling it is important to understand when each phase of the project occurred. After Phase A, the baseline phase, subsequent phases are compounding, or they build upon one another. Phase B is the introduction, the monkeys have been introduced when Phase C occurs, they continue to have access to each other overnight after the birds are introduced, Phase D, and conditions remain the same when the birds were removed returning to Phase C. The birds had to be removed after a period of 4½ days. On the morning of the fourth day that the birds were present Monkey 2 attacked one of the birds. He climbed up to the top of the enclosure and grabbed the bird and clawed at its face. The next day he did the same thing to another bird. After the second occurrence, the birds were removed from the enclosure and the study essentially returned to Phase C. The timeline for this project lasted for a total of five weeks.

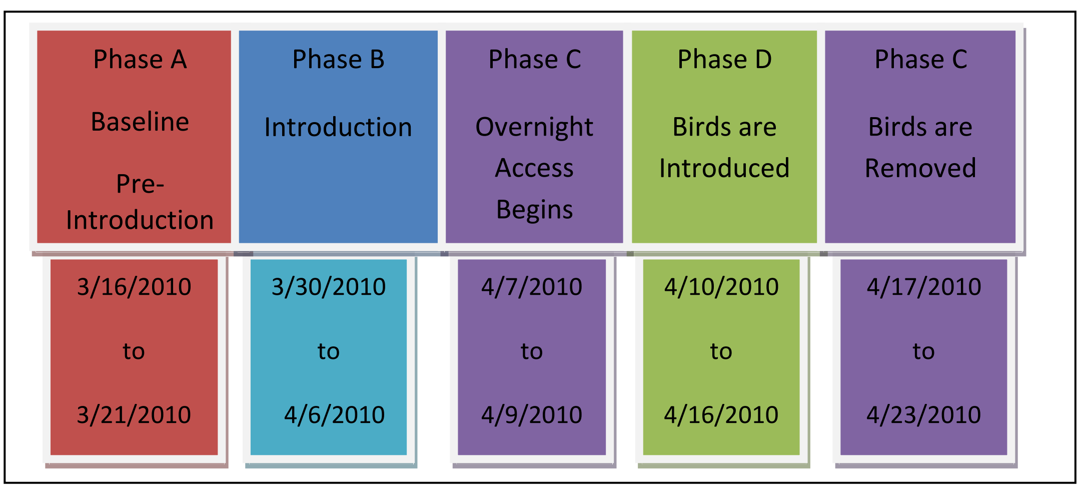


Figure 2. Introduction Timeline by Date.

At the beginning of this thesis I hypothesized that 1) the frequency of stressful behaviors will increase immediately after the introduction and will fall towards the end of the study; 2) salivary cortisol levels will increase immediately following the introduction and will fall towards the end of the study; and 3) there will be evidence of a correlation between stressful behaviors and higher levels of salivary cortisol. To address the outcome of each hypothesis I will focus on each research question that was asked and describe in more detail how the observed data relate to the anticipated results.

Question 1

What behavioral responses occur when two howler monkeys are introduced into the same exhibit at a zoo?

For each behavior that was documented I was looking for general trends of increases or decreases in frequency after the baseline phase (A) of the study. The behavioral frequencies represented by the following series of graphs are a compilation of the frequency observed for each focal period in the mornings and the afternoons. Each point on the graph is the average frequency of 10 randomly selected focal periods in the mornings and 10 randomly selected focal periods in the afternoons. The first three data points on each graph represent the baseline phase (A) of the study.

Allogrooming and Autogrooming. The evidence provided by this study indicates that allogrooming and autogrooming are inversely related (Figures 3-6). For Monkey 1 the first observed instance of allogrooming occurred on the very first day of the introduction in the afternoon. The line graphs indicate that allogrooming begins to trend upward on 4/7/2010, the day after the two monkeys had access to each other overnight. The four macaw parrots were reintroduced into the exhibit on 4/10/2010 and were removed on 4/16/2010. The highest average frequency of allogrooming that occurred during the focal periods of Monkey 1 happened after the birds were removed from the exhibit, and towards the end of the study. There is no clear trend observed for the behavior of allogrooming for Monkey 2. The first instance occurred on 4/6/2010 in the afternoon prior to Phase C of the study. The next instance came on the morning after Phase C of the study, and was not observed again until 4/22/2010. .

With respect to autogrooming in Monkey 1, the general trends indicate a decrease in average daily frequency after the initial baseline Phase (A) of the study in both the mornings and afternoons. Prior to the introduction autogrooming was observed everyday Monkey 2 was watched. Monkey 1 was more likely to autogroom herself than Monkey 2. In fact, Monkey 2 was not observed autogrooming after the baseline period. Once the male, Monkey 2, had someone to groom for him, he no longer did it himself. This makes sense because in free-ranging populations dominant males rarely if ever autogroom or even allogroom. Monkey 1 was observed allogrooming on Day one of the introduction, while Monkey 2 did not groom Monkey 1 until the sixth day of the introduction. Allogrooming for the male was rare. For the female, Monkey 1, the frequency of allogrooming began to increase following Phase C of the introduction. Having access to one another overnight appears to have strengthened the bond between the two monkeys.

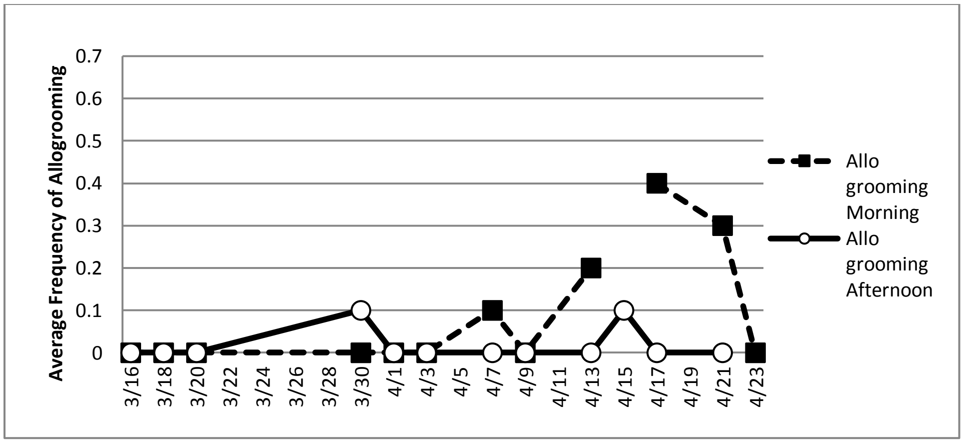


Figure 3. Monkey 1 Average Frequency of Allogrooming by Date.

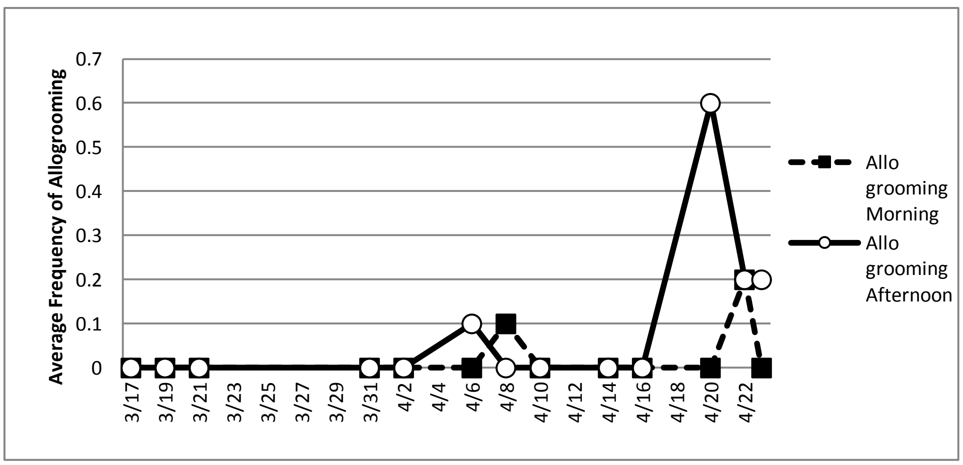


Figure 4. Monkey 2 Average Frequency of Allogrooming by Date.

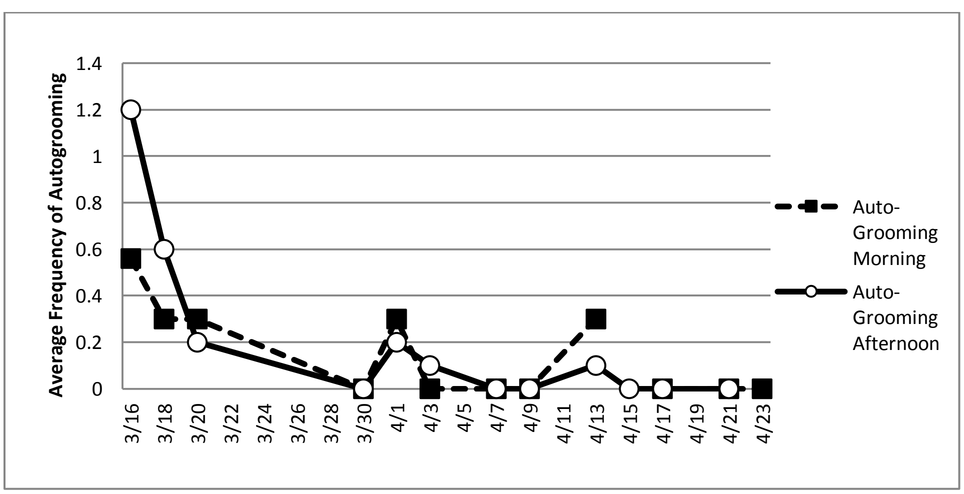


Figure 5. Monkey 1 Average Frequency of Autogrooming by Date.

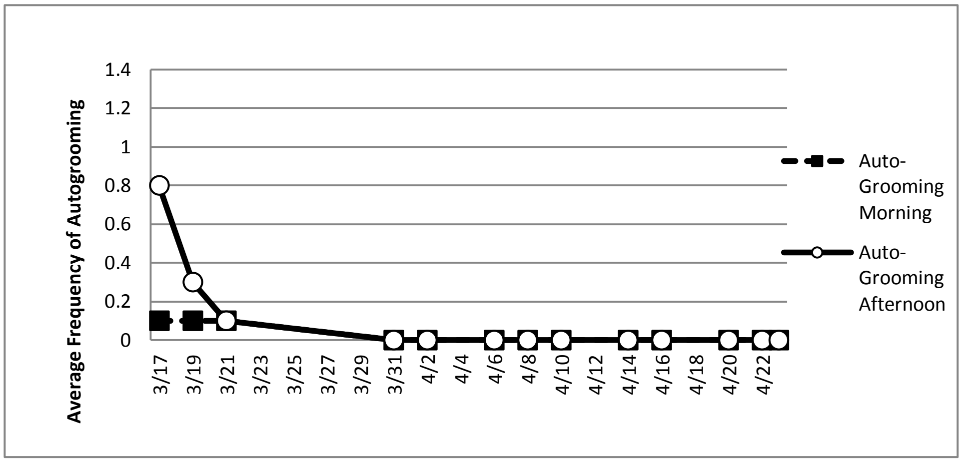


Figure 6. Monkey 2 Average Frequency of Autogrooming by Date.

Asleep or Awake. The differing phases of the introduction did not directly impact the amount of time spent asleep or awake (Figures 7 and 8). Monkey 1 did appear to be awake more frequently on the morning of the introduction, which is not surprising due to the presence of a new roommate. Both Monkey 1 and Monkey 2 exhibited patterns for sleep that have been typically noted in free-ranging populations (Kinzey 1997). In general they slept or rested over 50% of the time and were more active in the afternoons when the weather was warm.

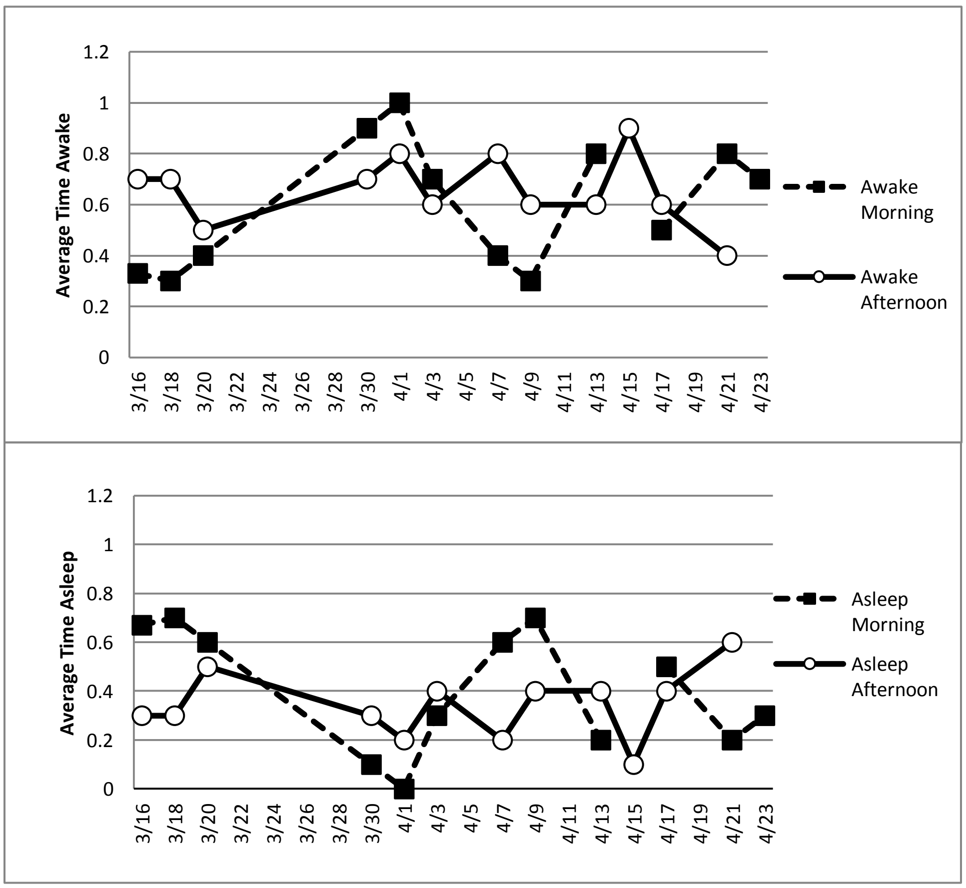


Figure 7. Monkey 1 Average Frequency of Time Spent Asleep by Date.

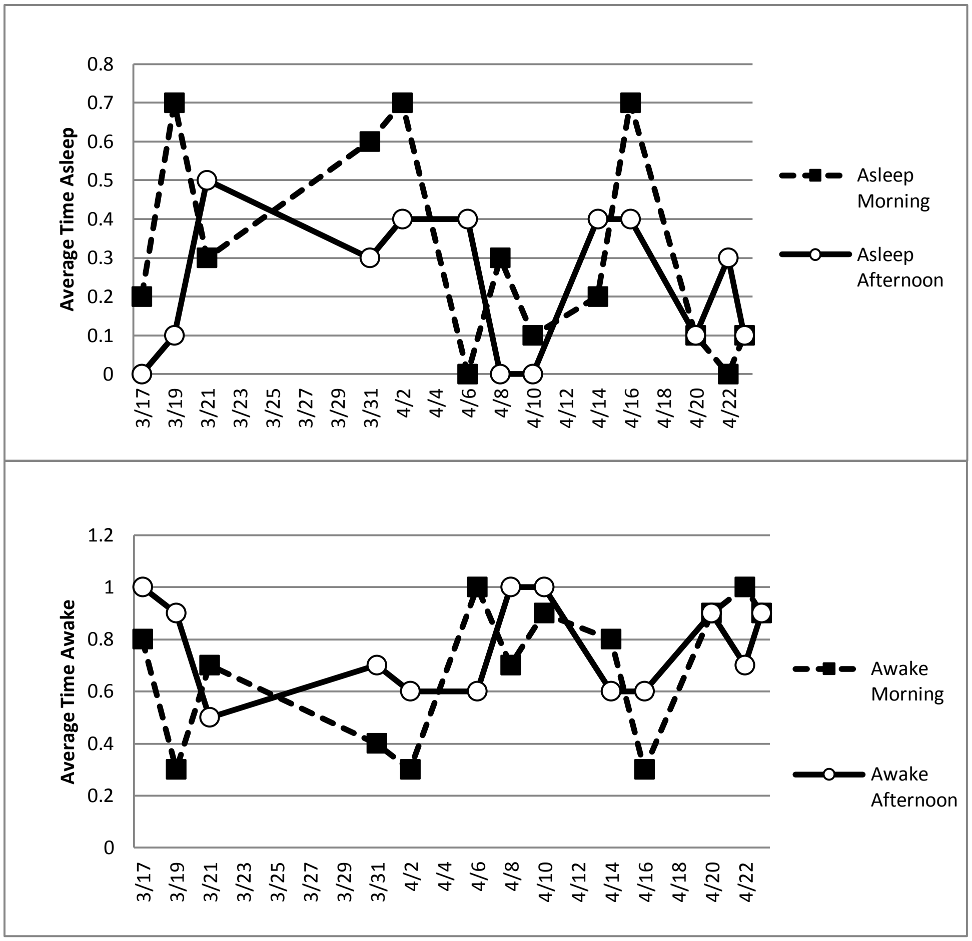


Figure 8. Monkey 2 Average Frequency of Time Spent Asleep and Awake by Date.

Hitting and Biting. These were the most aggressive behaviors for which the monkeys were monitored. When I hypothesized that stressful behaviors would rise following the introduction, these were the ones I had particularly in mind. For Monkey 1 this hypothesis was found to be false. She was observed hitting Monkey 2 on only one occasion, and it was late in the introduction period, Phase D (Figure 9). Monkey 1 bit Monkey 2 on two separate days towards the end of the introduction (Figure 11).

While both monkeys were generally very passive, Monkey 2 was more aggressive of the two. This is most likely due to the fact that he is male and much older than Monkey 1. Monkey 1 hit only once. On this day, Monkey 1 was attempting to rest on her back in the sun; Monkey 2 was trying to engage her sexually and she hit him. Monkey 2 was observed hitting Monkey 1 on three separate days (Figure 10). The first time he hit her was on Day 2 of the introduction. Things heated up when he took some grass up to their normal resting spot on a limb. Monkey 1 came over to Monkey 2 and began smelling him; she got very close. I believe he thought she was trying to steal his piece of grass and he hit her, not hard, but aggressively. The female backed off momentarily then began smelling him again, at which point he hit her again. This time she moved away and they stayed separate for the remainder of the afternoon.

The second time he hit her was the first day he was observed following Phase C of the introduction, again she got too close to him. The third time he hit her coincided with the first day of Phase D of the introduction, when the birds were brought into the exhibit, there also was a large crowd in the zoo that day. Monkey 2 attempted to engage Monkey 1 in sexual behaviors and he hit her as she moved away. The hitting behavior is the only behavior that appears to be related to the differing conditions of the introduction, each incidence occurred after Phases B, C, and D.

Monkey 2 was observed biting the other monkey on two occasions (Figure 12). Both times were relatively late in the study; the first occurrence was during Phase D, the birds were present. This is interesting because the most aggression displayed by Monkey 2 was directed at the birds. He attacked two of them on back-to-back days, 4/15/2010 and 4/16/2010.

After the second attack the birds were removed from the exhibit for their safety. Young (1982) observed the aggressive interaction between a troop of howler monkeys and turkey vultures, but there was no physical contact made between the two species.

The monkeys in Young's (1982) study became very vocal and assumed a rigid posture, but did not attack. Having read that study, I was surprised by the actions of Monkey 2 toward the birds; however, Monkey 2 was born in captivity, had never been housed with birds before, and was just beginning to get comfortable with his new surroundings. Ultimately Monkey 2 was more aggressive towards the birds than he was to Monkey 1. Although hitting coincided with the differing phases of the introduction, biting occurred only towards the end, therefore Monkey 2's exhibition of these behaviors fails to support my hypothesis that the frequency of stressful behaviors will increase immediately after the introduction and will fall towards the end of the study.

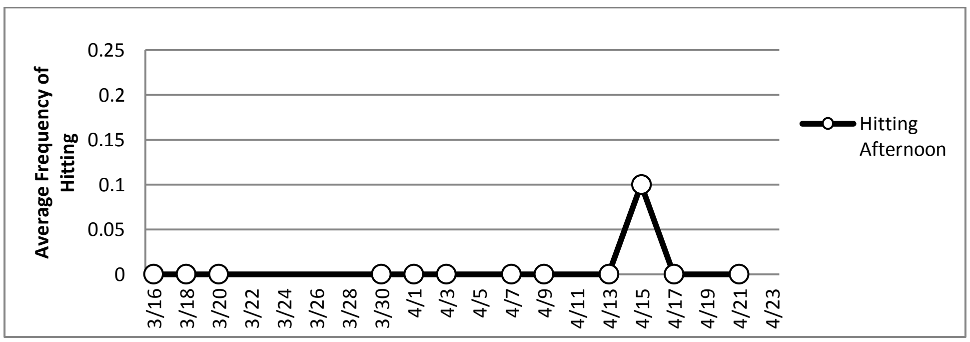


Figure 9. Monkey 1 Average Frequency of Hitting by Date

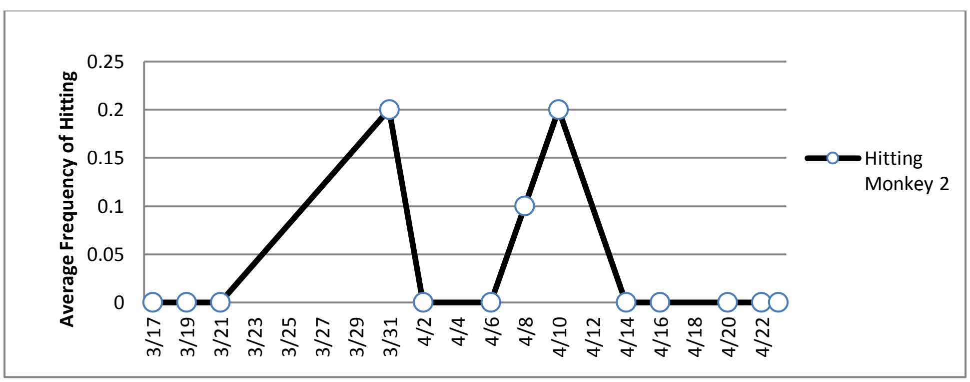


Figure 10. Monkey 2 Average Frequency of Hitting by Date

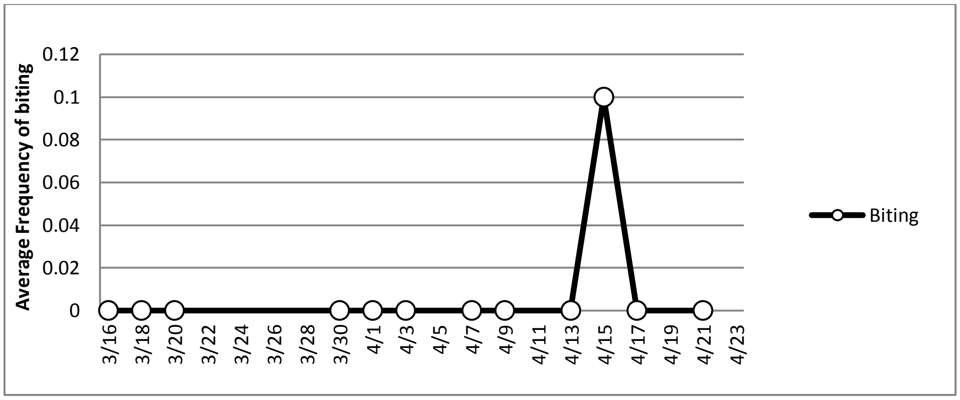
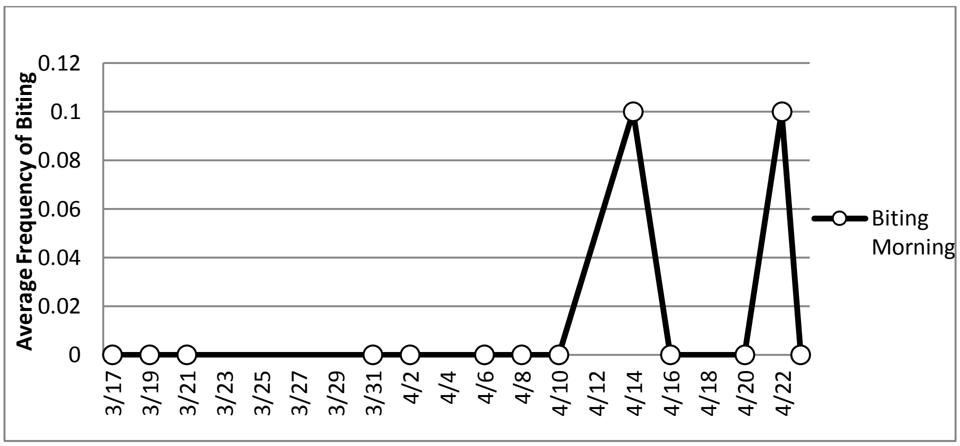


Figure 11. Monkey 1 Average Frequency of Biting by Date.



Figue 12. Monkey 2 Average Frequency of Biting by Date.

Play. Monkey 1 was observed playing once prior to the introduction and on three separate occasions post-introduction (Figure 13). One instance of play was observed during the baseline phase (A) of the study; it occurred on 4/20/2010 in the afternoon. Monkey 1 was observed playing with Monkey 2 on two occasions during phase C of the study; the play occurred at some stage in the morning on 4/13/2010 and also during the afternoon on 4/15/2010. Before the introduction she was observed playing with a rope, the instances after the introduction were attempts to engage Monkey 2 in play; the female would climb around the male, darting and weaving and touching his face. Monkey 2 was nonresponsive to these attempts, but Monkey 1 still tried. Monkey 2's aversion to play was likely due to his old age and disabled body. On no occasion did Monkey 2 attempt to initiate play or respond to Monkey 1's attempts. There is no relationship between play and the phases of the introduction.

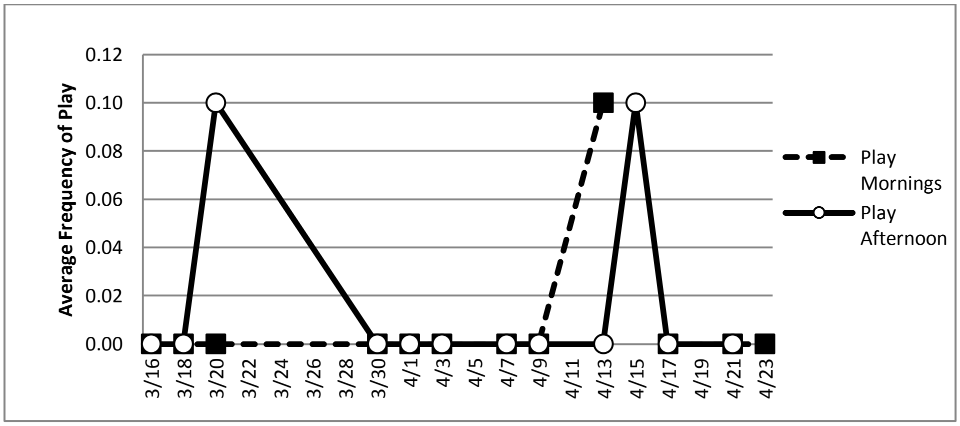


Figure 13. Monkey 1 Average Frequency of Play by Date.

Touching. This behavior is one of the few that appears to be affected by the changing phases of the introduction. In general, touching increased over the course of the introduction. Monkey 1 began touching Monkey 2 with the hand on Day 1 of the introduction in the afternoon. The average frequency peaked on 4/3/2010, the fifth day of the introduction. The frequency of touching decreased in both the mornings and afternoons after phase C of the introduction once the monkeys were allowed access to each other overnight. The frequency of touching rose again after the birds were introduced (Phase D). It is interesting that prior to Phase C (overnight access allowed), Monkey 1 touched Monkey 2 more frequently (Figure 14). After they were allowed access to each other at night time, Monkey 1 touched less often, and the frequency which Monkey 2 touched increased. To distinguish from grooming acts, these types of touches involve pats on the back, or caresses of the face, mainly touches with the fingers and hands. For Monkey 2, touches rose steadily until the introduction of the birds (Phase D), where they stopped almost entirely (Figure 15). Touching was not seen on the first day after the introduction that Monkey 2 was observed. The frequency began to trend upward as Phase C approached and rose again after Phase C was initiated. The frequency of touching dropped sharply once the birds were introduced into the exhibit (Phase D) and rose again after they were removed (return to Phase C). Monkey 2 was very interested in the birds, and followed them around for a couple of days before he attacked. I believe the decrease in his touching is simply related to his being distracted by the presence of the birds. When Monkey 1's touching decreased, it was following the first night they spent together. Phase C is an important point in the study because I believe that to be the point where Monkey 2 became the dominant animal.

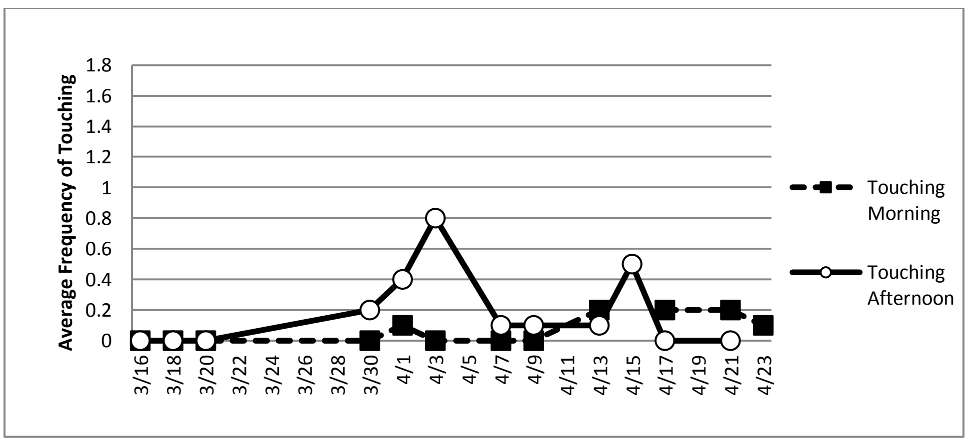


Figure 14. Monkey 1 Average Frequency of Touching by Date.

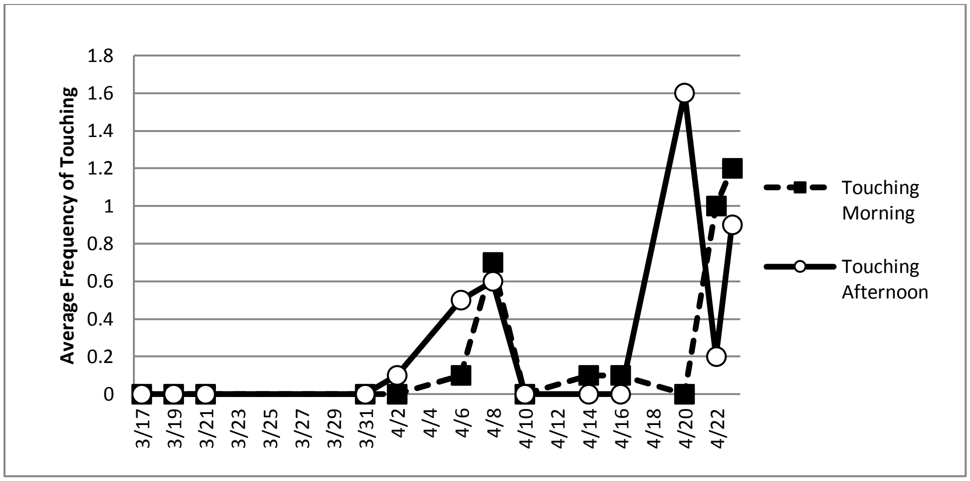


Figure 15. Monkey 2 Average Frequency of Touching by Date.

Smelling. There is no evidence that the frequency of smelling each other was affected by any phase change during the introduction by either monkey (Figures 16 and 17). The behavior of smelling occurred nearly every day post introduction. The highest frequency of Monkey 1 smelling or sniffing Monkey 2 occurred in the afternoon of 4/15/2010. For Monkey 2, the first instance occurred in the afternoon on the first day of Phase D and did not occur again until a few days after the birds were removed from the exhibit. The female monkey (Monkey1) was the more frequent smeller. This could be due to female mammals in general having a keener sense of smell than their male counterparts; however there really is no evidence to assign a cause for the phenomenon (Doty et al. 1985). Perhaps the female simply liked the way the male smelled.

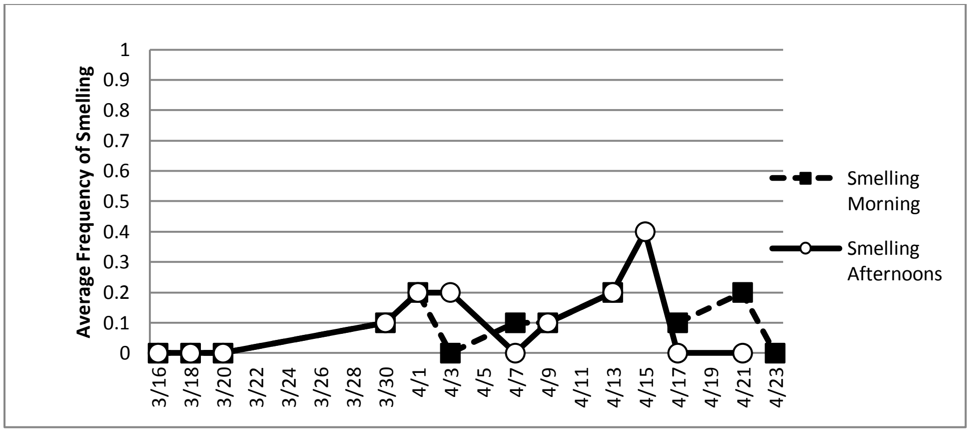


Figure 16. Monkey 1 Average Frequency of Smelling by Date.

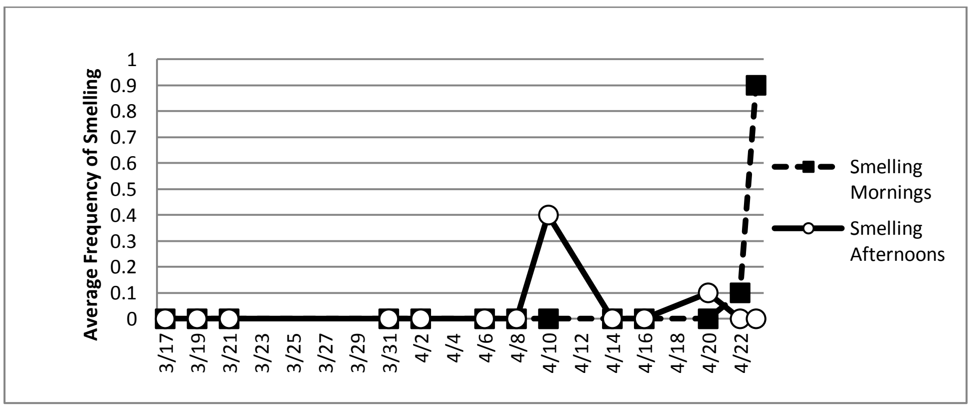


Figure 17. Monkey 2 Average Frequency of Smelling by Date.

Degree of Movement. Movement was measured by the degree to which they moved on the ground, climbed around the enclosure, twitched, or remained still. No pattern emerges for degree of movement across the introduction; however it is of interest when the monkeys moved the most. For Monkey 1, her degree of movement spiked on day one of the introduction (Figure 18); Monkey 2's movement peaked the day the birds were introduced (Figure 19). This indicates that Monkey 2 was more disturbed by the presence of the birds than he was another monkey. Monkey 1 was more disturbed by the presence of another monkey than she was by the birds that had been her exhibit mates for years.

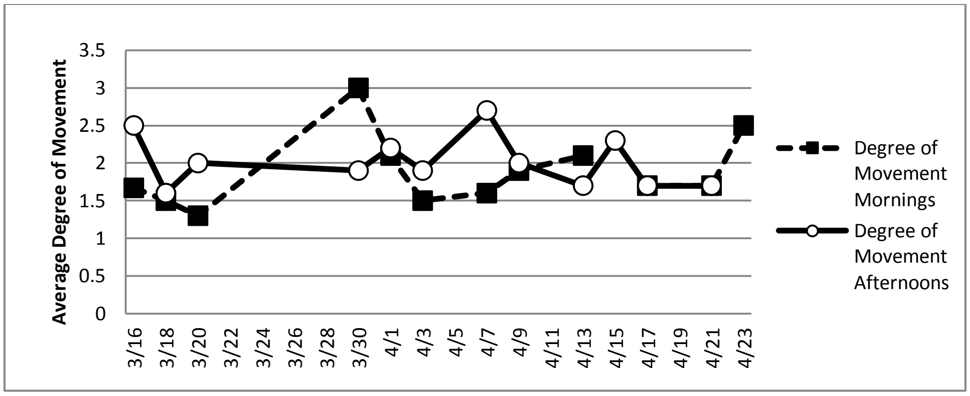


Figure 18. Monkey 1 Average Frequency of Degree of Movement by Date.

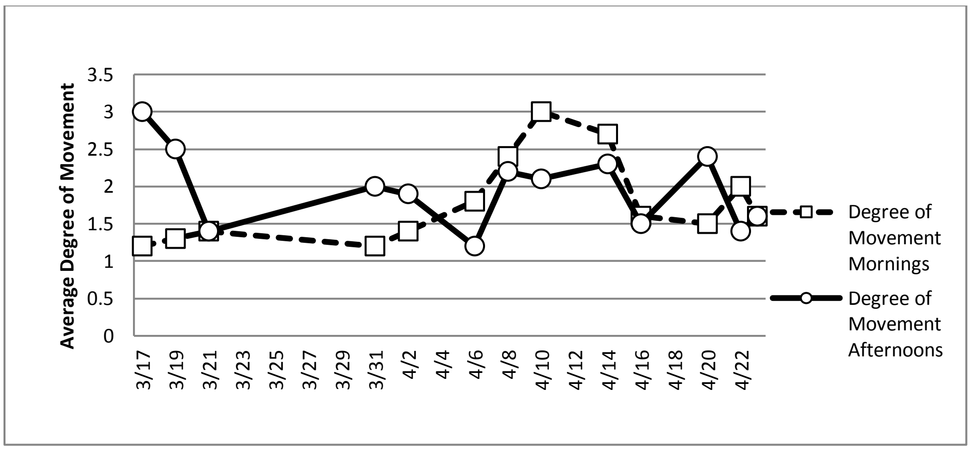


Figure 19. Monkey 2 Average Frequency of Degree of Movement by Date.

Climbing. Monkey 1 climbed more frequently post-introduction, the highest frequency came on Introduction Day 1 (Figure 20). Monkey 2 also climbed more frequently post-introduction (Figure 21). His highest frequency came in the afternoon on Day 1 of the baseline phase, but I believe this was due to my presence near his afternoon holding area. There was nowhere I could go where I could not be seen, and he moved around mostly to check me out. After that initial day he climbed a lot less. Monkey 2's next peaks in climbing came during Phase D of the study. At this time he climbed around a lot checking out the birds, most noticeably the day before he attacked for the first time. Obviously climbing goes along with degree of movement; Monkey 1 climbed most frequently on the day of the introduction (Phase B) and Monkey 2 climbed most frequently during Phase D.

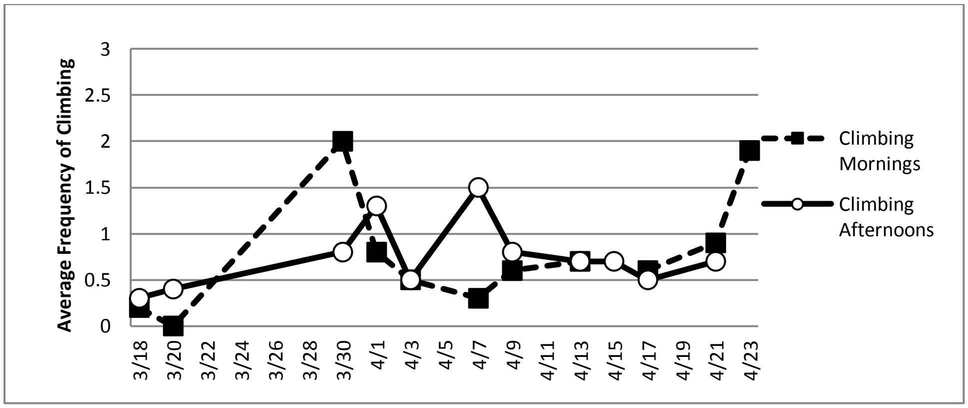


Figure 20. Monkey 1 Average Frequency of Climbing by Date.

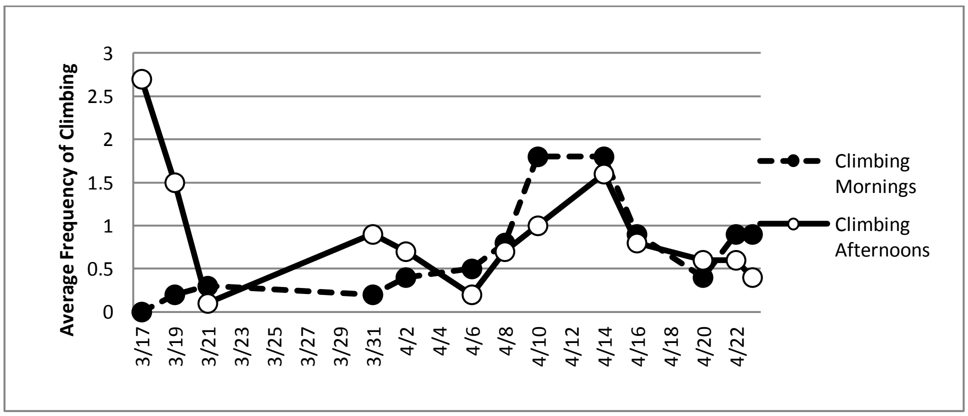


Figure 21. Monkey 2 Average Frequency of Climbing by Date.

Vulval and Clitoral Displays. The vulval display essentially serves as a caution light. It is employed by females when they are signaling males to either stop, retreat, or slow down (Jones 1983). Monkey 1 committed vulval displays on three separate afternoons towards the end of the study (Figure 22). There was no incidence of vulval display witnessed during the mornings. This involved lifting her tail and showing her backside to Monkey 2. At this point he had been following her around quite a bit, and I believe she simply had grown tired of it. Clitoral displays occurred more frequently beginning on Day 3 of the introduction (Figure 23). Female howler monkeys possess a clitoris that protrudes when it becomes erect. This is a behavior that indicates subordination (Jones 1983). It was most often seen during episodes of oral sex and foreplay performed by Monkey 2. There is no trend present for clitoral display since it was a fairly common occurrence throughout the introduction; the changing phases had no influence on the behavior.

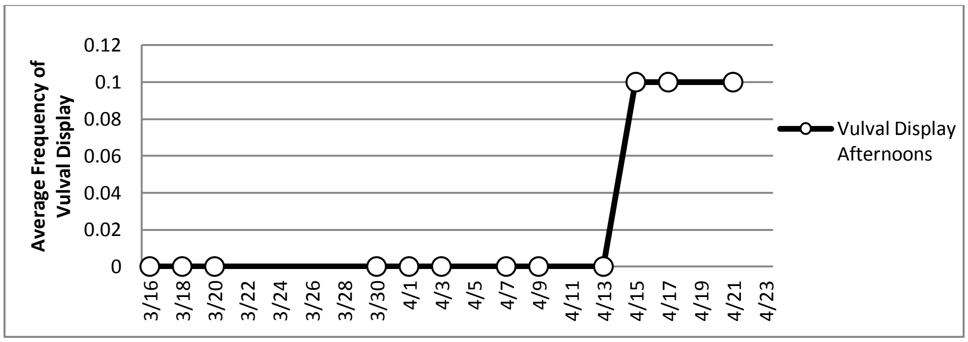


Figure 22. Monkey 1 Average Frequency of Vulval Display by Date.

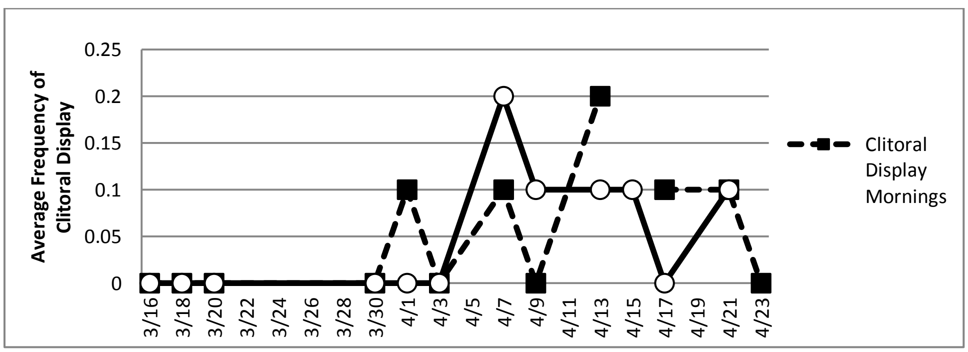


Figure 23. Monkey 1 Clitoral Display by Date.

Copulation. The lone act of copulation occurred in the afternoon on Monkey 1's focal day (Figure 24). It goes without saying that both monkeys were involved. The act of copulation is directly related to Phase C of the study. It occurred during the day after they were allowed access to each other overnight. Up until this point zoo workers were not even sure that Monkey 2 was capable of copulating due to his spinal dysplasia and bent over posture. It is worth mentioning that simply because this was the only time that

I observed intercourse does not mean that it is the only time that it happened. I was lucky to have caught the act because it occurred at the beginning of a focal period.

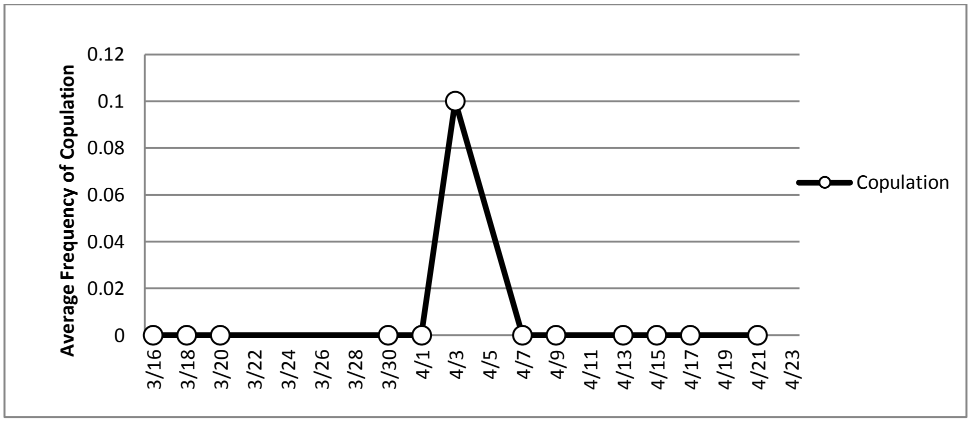


Figure 24. Average Frequency of Copulation by Date.

Howling and Grunting. In most general terms, howling increased with time over the course of the study and grunting decreased with time over the study (Figure 25 and 26). This was true for both animals. However, I do not want to read too much into this because grunting occurred more often when it was cold, and temperatures warmed up over the course of the study. The bouts of howling were observed during rainy weather and whenever a weed eater could be heard on the premises. Therefore, the phases of the introduction have little to do with the trends observed. Residents that live near the zoo informed me that the monkeys can be heard howling most mornings very early, but this is before the zoo is open and I was not privy to it. When the monkeys did howl it was instigated by the male, and the female joined in chorus with him. In addition to the morning wake-up roar that alerts the forest to a howler group's location, free-ranging populations have been observed roaring during rainstorms, after hearing a loud noise, when under threat of predation, after the discovery of the death of offspring, and as a result of many other potential stressors (Altman 1959). The howling observed happened as a response to varying stimuli, notably loud noises such as thunder and a weed eater.

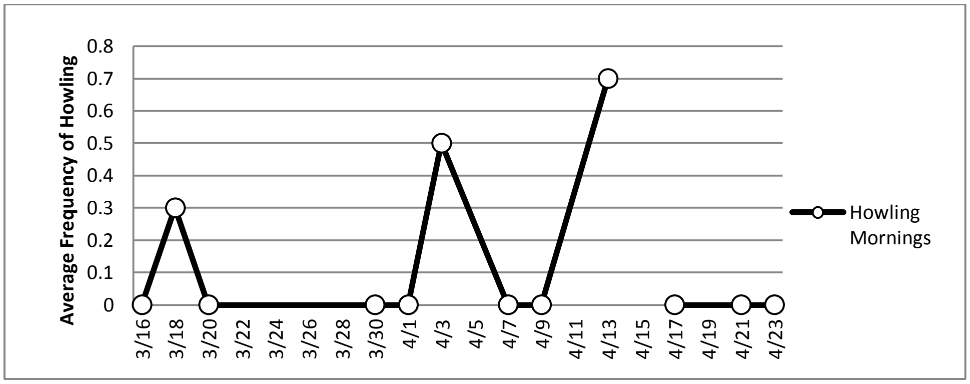


Figure 25. Monkey 1 Average Frequency of Howling by Date.

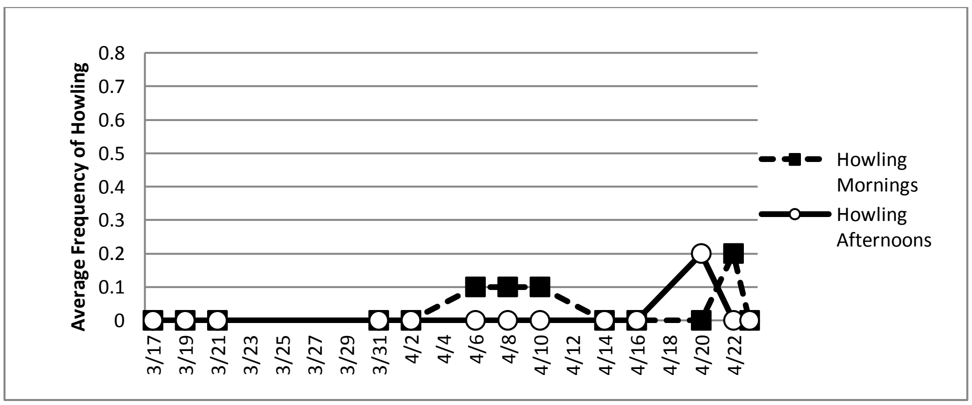


Figure 26. Monkey 2 Average Frequency of Howling by Date.

Grunting is another type of howler vocalization and is often referred to as a bark. This was the most frequent vocalization that was heard, and was observed more often in Monkey 2 than in Monkey 1. Monkey 1 was not observed grunting at all prior to the introduction (Figure 27). The first time Monkey 1 is heard grunting is on day one of the introduction. The average frequency of grunting continually rose and fell over the course of the introduction. There was a clear increase in both mornings and afternoons post baseline phase (A). In general, Monkey 2 grunted less frequently as the study went on

(Figure 28). There was no clear pattern for grunting associated with the phases of the introduction.

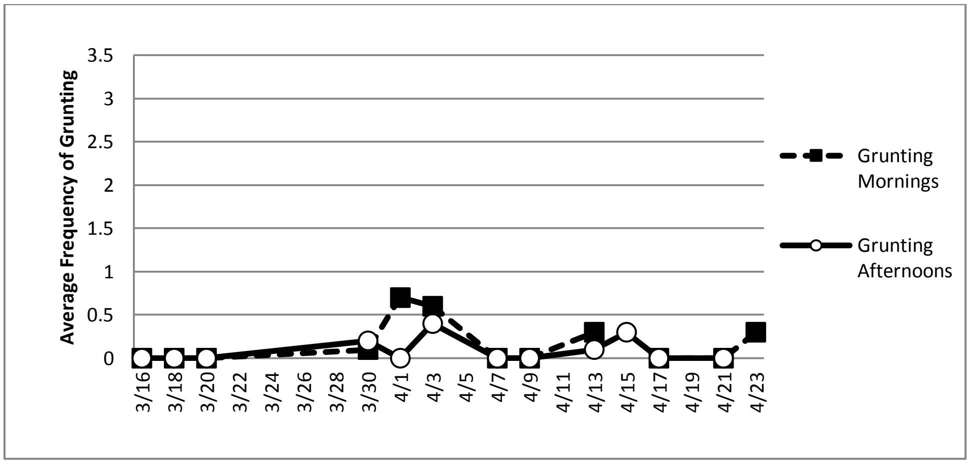


Figure 27. Monkey 1 Average Frequency of Grunting by Date.

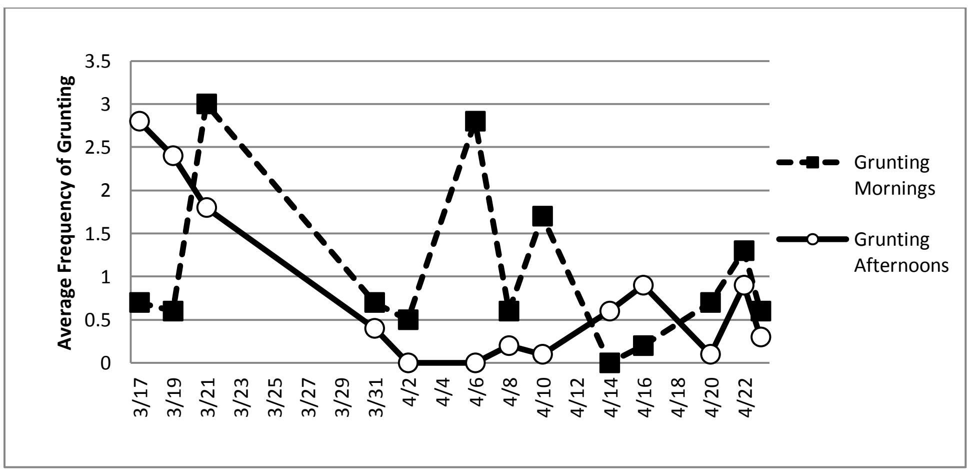


Figure 28. Monkey 2 Average Frequency of Grunting by Date.

Eating. There are no patterns associated with eating as related to various stages of the introduction (Figures 29 and 30). The monkeys were given breakfast on exhibit each morning, consisting of primate biscuits, vegetables and leafy greens, and generally ate as soon as they got outside. Monkey 1 was more likely to forage on leaves and grass in the afternoons. This was most likely due to her being more mobile and having the ability to reach through the fence to grab leaves off her favorite bushes. Monkey 2 began to forage on these leaves later in the study, but there were bushes that she had access to that he did not. Monkey 1 had this ability to swing the fence backwards and forwards to gain momentum towards bushes outside of the exhibit. Monkey 2 simply was not sufficiently mobile enough to do this.

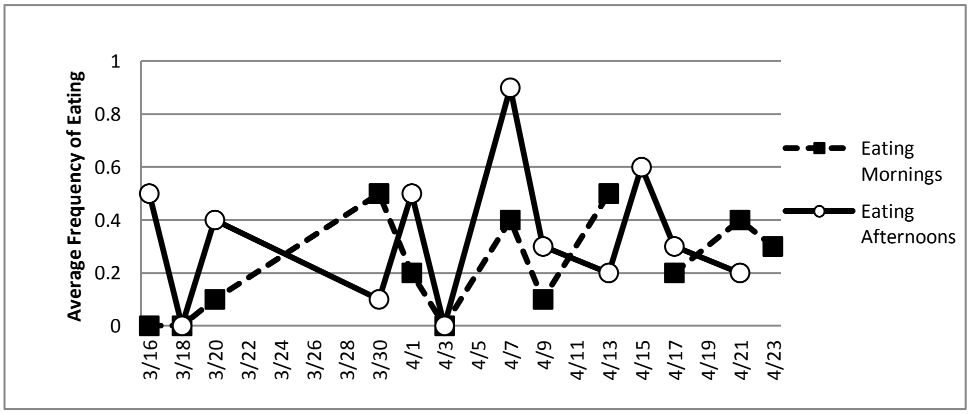


Figure 29. Monkey 1 Average Frequency of Eating by Date.

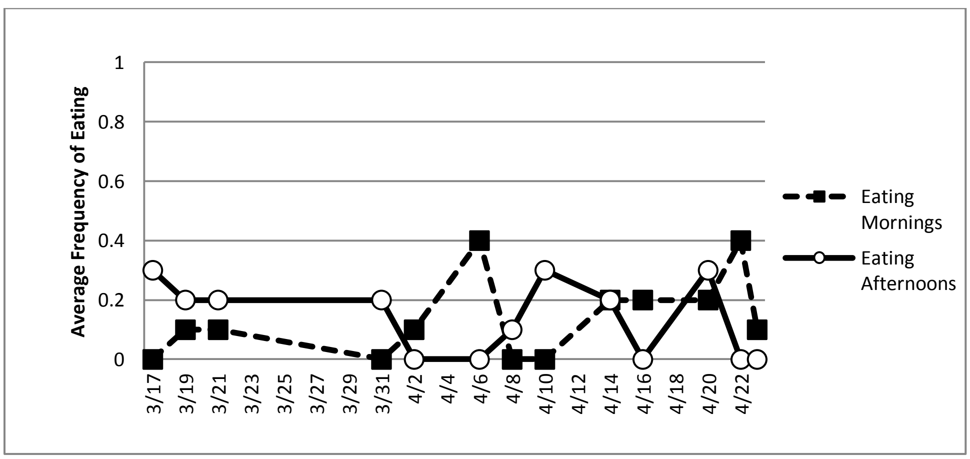


Figure 30. Monkey 2 Average Frequency of Eating by Date.

Yawning. There is no trend up or down for the prevalence of yawning for either monkey when comparing post-introduction to baseline phase (A) (Figures 31 and 32). For Monkey 1, this behavior was more likely to occur in higher frequencies in the afternoon. It is worth pointing out that for Monkey 2, yawning spiked in both the mornings and afternoons after Phase C of the introduction. Perhaps he was more tired because they had access to each other overnight, but there is no way to know for sure. I included this behavior as one to watch in order to see if their patters of yawning might syncopate (did one monkey yawning cause the other monkey to yawn), but they did not.

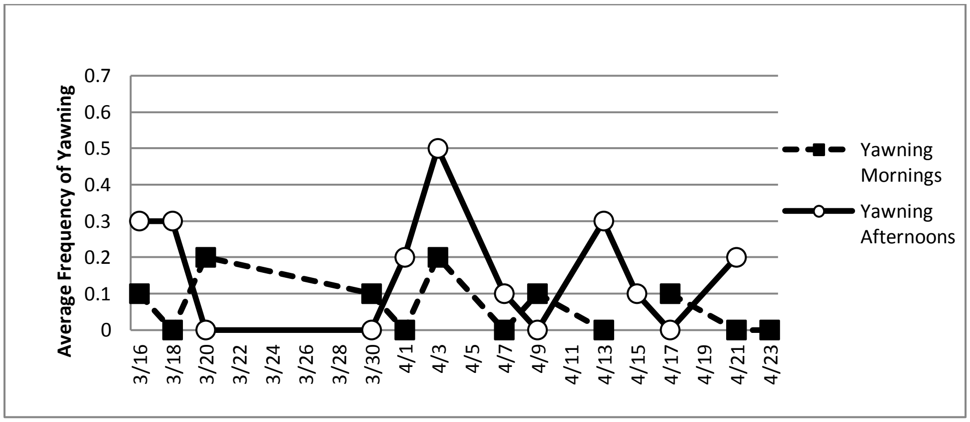


Figure 31. Monkey 1 Average Frequency of Yawning by Date.

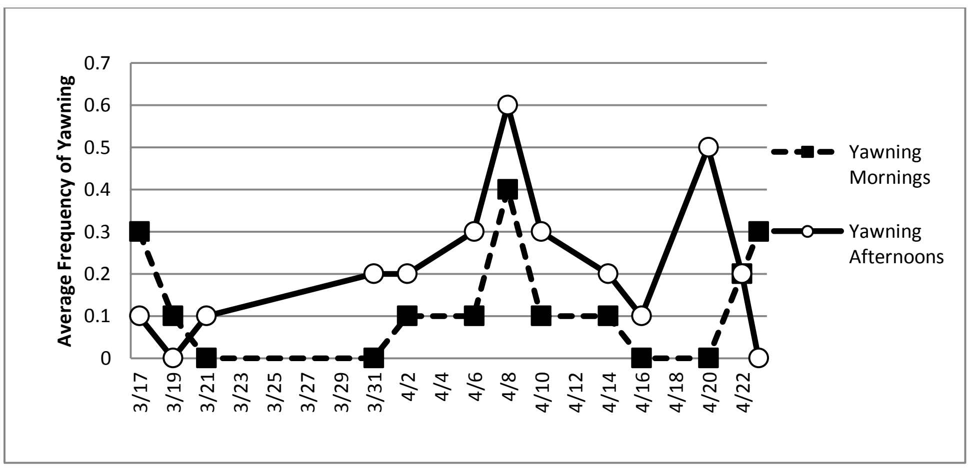


Figure 32. Monkey 2 Average Frequency of Yawning by Date.

Scratching. There is no pattern or trend associated with scratching for either monkey (Figures 33 and 34). If they itched they scratched and it is as simple as that. I included this behavior because it occurred most often; the monkeys scratched during the majority of the focal periods. Including this behavior also helped to quantify the autogrooming behavior, which involved ruffling of the fur and picking at it, as opposed to a momentary scratch.

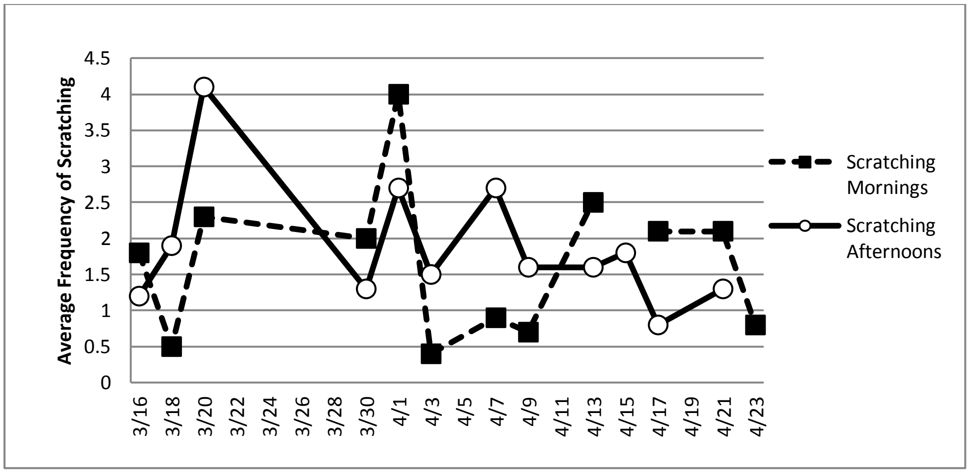


Figure 33. Monkey 1 Average Frequency of Scratching by Date.

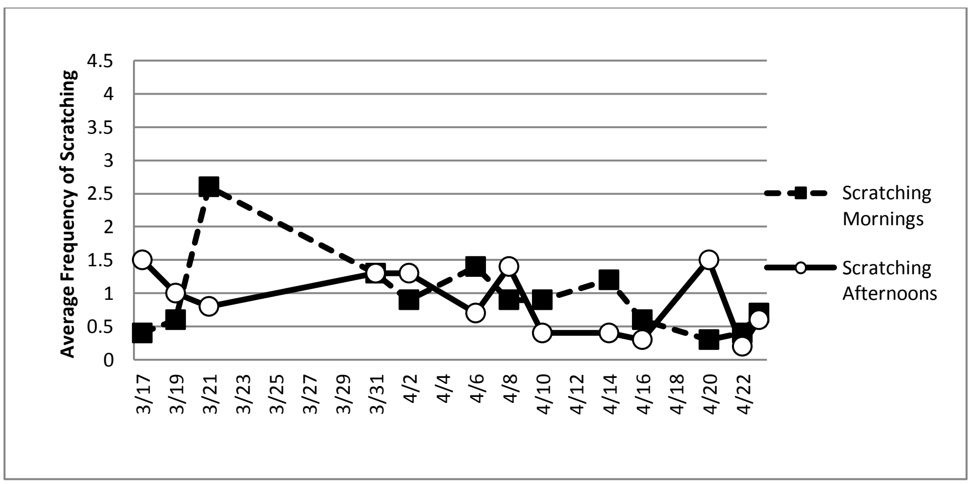


Figure 34. Monkey 2 Average Frequency of Scratching by Date.

Body Tremors. There is no pattern related to body tremors and introduction phases (Figure 35). Body tremors were only observed in Monkey 2 and were related to cold weather. Monkey 2 had never been housed in an outdoor exhibit and was not used to the cold. The occurrence of a couple of body tremors on the day Phase D was instituted is most likely due to coincidence.

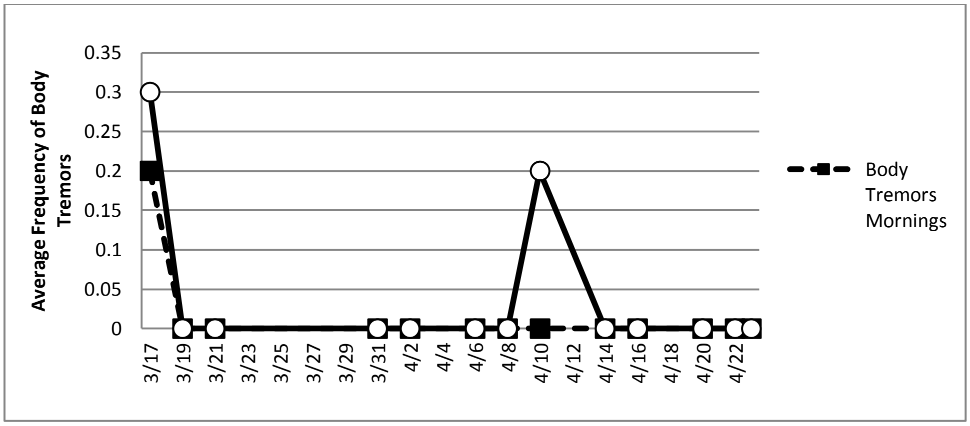


Figure 35. Monkey 2 Average Frequency of Body Tremors by Date.

Supplantation. This behavior involved physically making the other monkey move, uprooting them from a chosen spot. Monkey 2 displayed this behavior twice, once early in the introduction period and once towards the end (Figure 36). Monkey 1 did not display this behavior. The first supplantation incident happened the first time Monkey 2 hit Monkey 1. After he hit her, he forced her away from her spot. During the second instance, there was a lot of noise in the zoo (weed eater, train whistle), and Monkey 2 pushed at Monkey 1 and took her spot. These findings support what Jones (1983) found, namely that supplantations were usually instigated by males, and they were not likely to escalate to violence.

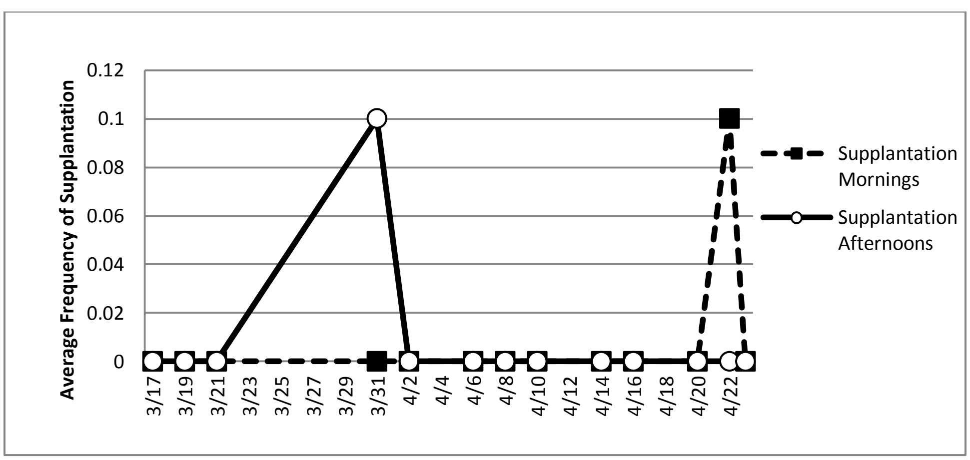


Figure 36. Monkey 2 Average Frequency of Supplantation by Date.

Chasing/Following. This is another behavior exhibited only by Monkey 2, the male. The frequency in general trended upwards over the course of the introduction, particularly after Phase D (Figure 37). This trend again supplements the disturbance incurred by Monkey 2 after the birds were introduced. It seemed like for awhile everywhere the female monkey went, the male would follow. What is interesting is that the frequency fell off in the last two afternoons Monkey 2 was observed for study. On Monkey 1's focal days during this time she was showing vulval displays to Monkey 2 in the afternoon, essentially giving him the back-off message. Monkey 2 ceasing to follow her around on those last two days indicates that he got the message.

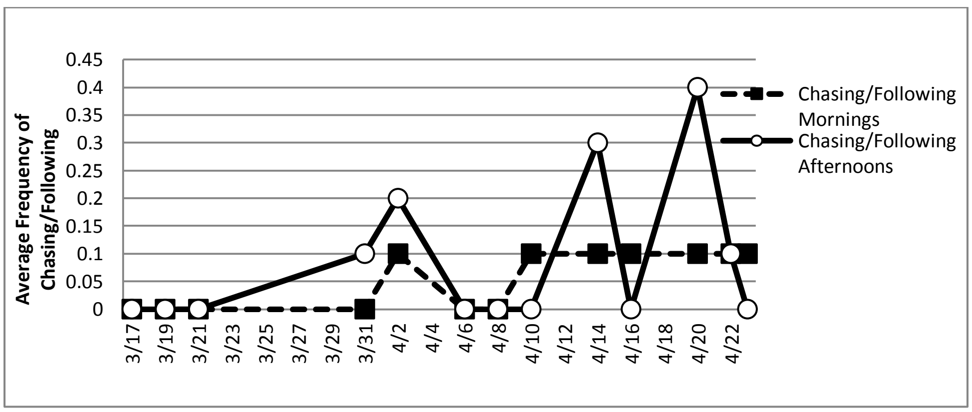


Figure 37. Monkey 2 Average Frequency of Chasing/Following by Date.

Of the behaviors that were monitored for, the frequencies of grooming, hitting, touching, climbing, copulation, and howling were found to be influenced by the changing phases of the introduction. I hypothesized that: The frequency of stressful behaviors will increase immediately after the introduction and will fall towards the end of the study. No evidence was found to support this hypothesis; however five behaviors were affected by the changing conditions of the study.

Question 2

How stressed are the animals at different stages of the introduction?

In order to answer this question I have looked at general trends in cortisol levels at different stages of the introduction for each monkey. The first three dates on each graph represent the baseline phase (A) or the pre-introduction.

Monkey 1 Cortisol Results

Cortisol levels were relatively stable for Monkey 1 throughout the study. The highest peak occurred on day one of the introduction (Figure 38). Monkey 1's cortisol peaks in both the mornings and the afternoons on 3/30/2010. Cortisol levels tended to be lower in the afternoons than in the mornings. See Table 1 for a detailed list of Monkey 1 cortisol levels, dates and collection times and a description of the phases of the study. The average baseline cortisol level for the mornings was $\mu = 1.99ug/dL$. The average morning cortisol level after the introduction was $\mu = 2.43ug/dL$. The average baseline cortisol level for the afternoons was $\mu = 0.485ug/dL$. The average cortisol level for the afternoons after the introduction was $\mu = 1.273ug/dL$.

The average cortisol levels in both the morning and the afternoon after the introduction were higher than the baseline average. This indicates that the introduction did affect the stress levels of Monkey 1. This is further substantiated by the fact that the highest cortisol levels in the morning and the afternoon occurred on day one of the introduction. Monkey 1's cortisol results support Hypothesis 2 that salivary cortisol levels will increase immediately following the introduction and will fall towards the end of the study.

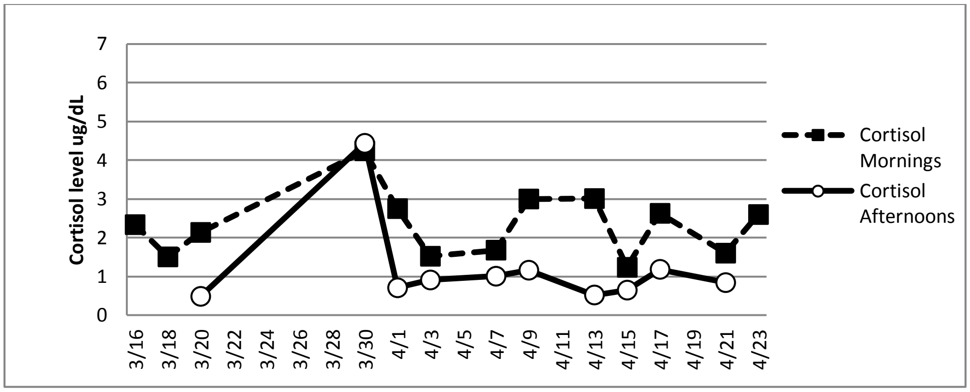


Figure 38. Monkey 1 Cortisol Level ug/dL by Date.

Table 1

Monkey 1 Cortisol Results

Sample ID	Result 1	Result 2	Mean (ug/dL)	Date	Swab Time	Project Phase
1-1	2.271	2.401	2.336	3/16/2010	10:02 a.m.	Baseline Phase A
1-2	qns	qns	qns			
1-3	1.464	1.544	1.504	3/18/2010	9:05 a.m.	
1-4	qns	qns	qns		3:15 p.m.	
1-5	2.115	2.154	2.135	3/20/2010	9:05 a.m.	
1-6	0.464	0.505	0.485		3:05 p.m.	
1-7	4.228	4.255	4.241	3/30/2010	9:40 a.m.	I-day 1 Phase B
1-8	4.339	4.530	4.435		3:10 p.m.	
1-9	2.625	2.875	2.750	4/1/2010	9:25 a.m.	I-day 3
1-10	0.684	0.728	0.706		3:15 p.m.	
1-11	1.544	1.495	1.520	4/3/2010	9:35 a.m.	I-day 5
1-12	0.891	0.925	0.908		3:05 p.m.	
1-13	1.624	1.732	1.678	4/7/2010	9:10 a.m.	I-day 7 Phase C
1-14	0.994	1.016	1.005		3:07 p.m.	
1-15	2.883	3.112	2.998	4/9/2010	9:10 a.m.	I-day 9
1-16	1.108	1.212	1.160		3:25 p.m.	
1-17	2.851	3.166	3.009	4/13/2010	9:05 a.m.	I-day 11 Phase D
1-18	0.493	0.549	0.521		3:05 p.m.	
1-19	1.294	1.173	1.234	4/15/2010	9:05 a.m.	I-day 13
1-20	0.610	0.681	0.646		3:25 p.m.	
1-21	2.507	2.750	2.628	4/17/2010	9:05 a.m.	I-day 15 Phase C
1-22	1.210	1.154	1.182		3:10 p.m.	
1-23	1.633	1.567	1.600	4/21/2010	9:15 a.m.	I-day 17
1-24	0.864	0.816	0.840		3:05 p.m.	
1-25	2.460	2.735	2.597	4/23/2010	9:15 a.m.	I-day 19

Note: qns=quantity not sufficient

Monkey 2 Cortisol Results. Monkey 2's cortisol graphs are more disjointed than Monkey 1's and therefore are more difficult to discern. See Table 2 for a detailed list of Monkey 2's cortisol levels, dates and collection times and a description of the phases of the study. There is no cortisol reading for Monkey 2 on the first day of the introduction, however his levels on introduction day two are within normal range. Monkey 2 exhibited the typical patterns of lower afternoon cortisol levels and higher morning cortisol levels. On the morning of 4/22/2010 cortisol reaches an abnormal peak (Figure 39). Upon the removal of this data point from the series, the next highest peak is found on 4/10/2010, day one of Phase D of the study (Figure 40). Cortisol peaks in both the morning and afternoon on this day (with the exception of the morning outlier from 4/22/2010).

Saliva samples were not taken from Monkey 2 on the first day of the introduction because he was not the focal animal. On the second day of the introduction, both his morning and afternoon cortisol levels were within range of the baseline. On 4/22/2010 Monkey 2 had an abnormally high cortisol level. For the purposes of this study, this level is considered an outlier. I have no substantiated reason why this level is so high; however Monkey 2 does have periodontal disease which may have led to the saliva sample being contaminated with blood, which would skew the results upward. During the first day the birds were introduced (Phase D), Monkey 2 had high cortisol levels peaking at 6 ug/dL in both the morning and afternoon. This suggests that the birds were a significant stressor. This is further substantiated by the fact that on two consecutive days, Monkey 2 attacked two different birds. The bird attacks were the most aggressive actions taken by Monkey 2. Fortunately the birds' injuries were not severe, and each parrot fully recovered. Both attacks occurred quickly after all the animals were released onto the exhibit.

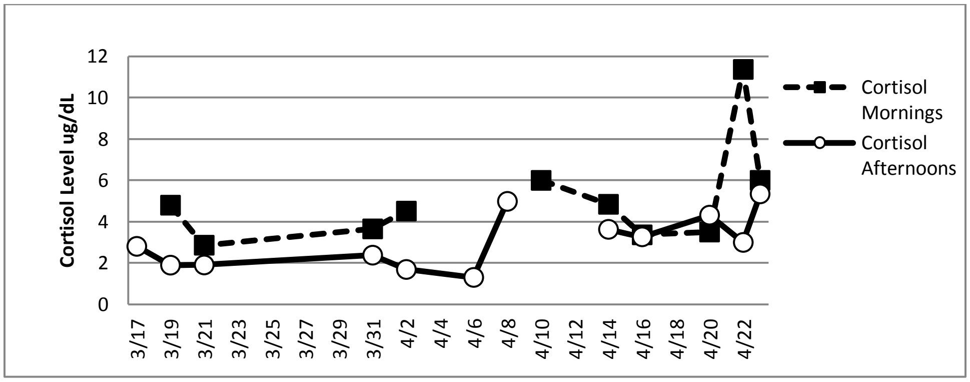


Figure 39. Monkey 2 Cortisol Level ug/dL by Date.

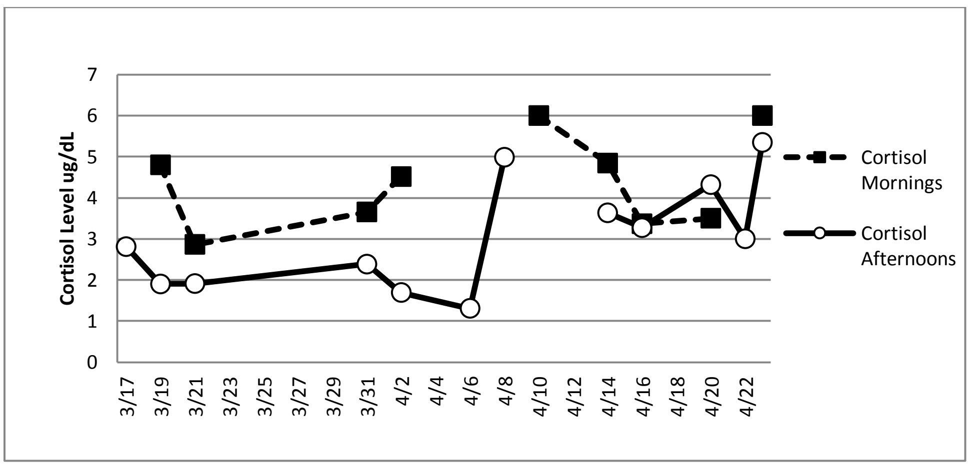


Figure 40. Monkey 2 Cortisol Level ug/dL Without Outlier by Date.

Table 2

Monkey 2 Cortisol Results

Sample ID	Result 1	Result 2	Mean (ug/dL)	Date	Swab Time	Introduction Sequence
2-1	qns	qns	qns	3/17/2010	9:15 a.m.	Baseline
2-2	2.750	2.867	2.809		3:05 p.m.	
2-3	4.809	qns		3/19/2010	9:10 a.m.	
2-4	1.979	qns			3:05 p.m.	
2-5	2.820	2.908	2.864	3/21/2010	9:10 a.m.	
2-6	1.834	1.987	1.910		3:05 p.m.	
2-7	3.488	3.820	3.654	3/31/2010	9:25 a.m.	Introduction Day 2
2-8	2.453	2.313	2.383		3:20 p.m.	
2-9	4.325	4.702	4.514	4/2/2010	9:35 a.m.	I-day 4
2-10	1.596	1.787	1.692		3:35 p.m.	
2-11	1.258	1.356	1.307	4/6/2010	2:55 p.m.	I-day 6
2-12	qns	qns	qns	4/8/2010		
2-13	4.783	5.186	4.985		3:25 p.m.	I-day 8
2-14	>6.0	>6.0	>6.0	4/10/10 qns to repeat	9:15 p.m.	I-day 10 (Birds Introduced)
2-15	qns	qns	qns		3:45 p.m.	
2-16	4.734	4.952	4.843	4/14/2010	9:10 a.m.	I-day 12
2-17	3.605	3.660	3.632		3:00 p.m.	
2-18	3.386	3.346	3.366	4/16/2010	9:10 a.m.	I-day 14 (2nd bird attack)
2-19	3.194	3.346	3.270		3:15 p.m.	
2-20	3.376	3.627	3.501	4/20/2010	9:10 a.m.	I-day 16
2-21	4.339	4.297	4.318		3:05 p.m.	
2-22	10.983	11.749	11.366	4/22/2010	9:15 a.m.	I-day 18
2-23	>3.0	>3.0	>3.0	qns to repeat	3:10 p.m.	
2-24	>6.0	>6.0	>6.0	4/23/10 qns to repeat	9:10 a.m.	I-day 19
2-25	5.131	5.561	5.346		3:05 p.m.	

Note. qns=quantity not sufficient

Monkey 2's cortisol charts are much less conclusive because he was more difficult to obtain a sample from. Monkey 1 was trained to submit a sample for a longer amount of time than Monkey 2 was, and there were a few times that Monkey 2 refused to submit a sample. Monkey 2's cortisol results do not support Hypothesis 2 that salivary cortisol levels will increase immediately following the introduction and will fall towards the end of the study. The study provides a good measure of how stressed the animals were at various stages of the introduction through salivary cortisol levels. Even though

Monkey 2's cortisol levels did not support Hypothesis 2, Monkey 1's levels did. The highest cortisol peaks for Monkey 1were recorded on the first day of the introduction. This indicates that the introduction invoked a physiological stress response, causing cortisol to flood into Monkey 1's bloodstream and subsequently into her saliva. Monkey 2's highest peak happened when the birds were introduced, indicating that Phase D had a direct impact on stress.

Question 3

What is the correlation, if any, between behaviors and hormones?

Each salivary cortisol level was compared with the mean frequency for the observed behaviors of 10 randomly selected focal periods each morning and afternoon, and a Pearson correlation value was determined to relate the variables X and Y. Pearson's r indicates the strength of a relationship with values ranging from 0.00 to ±1.00; where 0.00 indicates no relationship and ±1.00 shows a strong relationship (Healey 2007). For the purpose of this study Pearson correlations ranging between 0.00 - ±0.30 stand for no relationship, ±0.30 - ±0.60 stand for some relationship, and ±0.6 - ±1.00 stand for a strong relationship or correlation (Healey 2007). Significance levels will be set at $p \leq .05$.

Monkey 1. The Pearson's correlations for Monkey 1 were inconclusive. Only two behaviors, clitoral display and yawning, indicated that there may be a loose relationship with salivary cortisol; however they were not significant in a 2-tailed test. Based on this evidence I must conclude that none of the observed behaviors of Monkey 1 are indicators of stress (Table 3). The highest r value is for clitoral display where r= .349, but this value is not significant the .05 level. The next highest values for r are for vulval display and yawning where r=-.344 for each behavior; however neither of these behaviors are statistically significant. No other behaviors produced an r value above ± .30.

Table 3

Pearson's Correlation Between Behavior and Cortisol Levels for Monkey 1

Behavior	N	Pearson Correlation (r)	Sig. (2-tailed) (p)
Allogrooming	22	.218	.392
Autogrooming	22	.032	.886
Asleep	22	-.132	.558
Awake	22	.132	.558
Hitting	22	-.236	.290
Biting	22	.024	.917
Play	22	-.170	.450
Touching	22	-.228	.307
Smelling	22	-.084	.709
Degree of Movement	22	.234	.295
Climbing	22	.251	.260
Vulval Display	22	-.344	.117
Clitoral Display	22	.349	.111
Copulation	22	-.185	.409
Howling	22	.118	.600
Grunting	22	.170	.448
Eating	22	-.173	.441
Yawning	22	-.344	.117
Scratching	22	-.072	.750

Note. *=p≤.05, **=p≤.01, ***=p≤.001

Monkey 2. The Pearson's correlations for Monkey 2 were more conclusive than those of Monkey 1. As seen in Table 4 the amount of time spent asleep and the amount of time spent awake for Monkey 2 produced r values of -.350 and .350 respectively, but they are not statistically significant. Eating produced a r value of .339, but it was not significant. Supplantation and salivary cortisol produced a r value of .439 and a significance level of .041 which is significant at p ≤ .05, indicating that there is some relationship between the two variables. For the behavior of touching r=.494 with a significance level of .019 which is also significant. The behaviors of howling and biting produced the highest r values in the study and both are significant at $p \leq .01$. The r value for biting is .626 and the level of significance is .002; for howling r=.637 with a highly significant value of $p \leq .001$. These values indicate a strong relationship between the frequency of howling and increased cortisol levels and the frequency of biting and increased cortisol levels.

Table 4

Pearson's Correlation Between Behavior and Cortisol Levels for Monkey 2

Behavior	N	Pearson Correlation (r)	Sig. (2-tailed)
Allogrooming	22	.238	.287
Autogrooming	22	-.223	.318
Asleep	22	-.350	.110
Awake	22	.350	.110
Hitting	22	-.104	.646
Biting	22	.626	.002**
Play	-	-	-
Touching	22	.494	.019*
Smelling	22	.221	.323
Degree of Movement	22	.170	.450
Climbing	22	.090	.692
Copulation	-	-	-
Howling	22	.637	.001***
Grunting	22	-.066	.771
Eating	22	.339	.123
Yawning	22	.147	.513
Scratching	22	-.232	.299
Body Tremors	22	-.122	.589
Supplantation	22	.439	.041*
Chasing/Following	22	.105	.641

Note. *=$p \leq .05$, **=$p \leq .01$, ***=$p \leq .001$

Summary of Hormones and Behavior. Following what is generally known about non-human primates, Monkey 2, the male, exhibited more aggressive behaviors than the female. One of the aims of this study was to link damaging behaviors with stress and supplantation and biting actually did correlate. Supplantation is really more of a passive behavior that rarely escalates to damaging behaviors or physical violence. Biting definitely falls under the category of damaging behavior. For the behavior of touching, I believe that the correlation showed some relationship in Monkey 2 because he was not as prone to it as Monkey 1. I do not believe there is a true relationship between touching and stress. Howling was always instigated by Monkey 2, which is typical for howler monkeys. Monkey 2 always joined in with Monkey 1's howling; this may be the reason that howling did not correlate with cortisol levels in Monkey 1. The behaviors of supplantation, biting and howling support Hypothesis 3: there will be evidence of a correlation between stressful behaviors and higher levels of salivary cortisol. I cannot say that any of these behaviors are definitive indicators of stress due to the small sample size. The evidence provided by this study does warrant further investigation and future studies may provide more definite conclusions.

CHAPTER V

CONCLUSION

The howler monkey study documented the successful introduction of a male and female howler monkey into one exhibit at the Hattiesburg Zoo. The behavior of these captive primates generally is on par with that observed in their free-ranging cousins (Kinzey 1997). A typical day in the lives of these howlers included breakfast quickly after they were released on exhibit, followed by morning resting periods and naps. On warm and sunny days they could be seen lying on their backs basking in the sun. The afternoons were racked with more naps, and usually a foraging period. They would follow the sun across the exhibit; the favorite afternoon resting spot was on the opposite side of the exhibit from the favorite morning spot. Both monkeys were more mobile when the sun was shining and the weather was warm. On cold or rainy days they generally would huddle together. With the exception of Monkey 2 attacking a couple of the macaw parrots, these monkeys were extremely passive when it came to conflict. They exhibited ritualized behaviors such as vulval displays by the female and supplantation by the male to avoid aggressive interaction. These monkeys acclimatized rather quickly to each other, and still are getting along great.

Phase B of the study had a direct impact on a few behaviors. For Monkey 1 the frequency of touching, climbing, and the degree of movement reached their peaks at that point. Monkey 2 hit Monkey 1 for the first time during Phase B, the initial introduction period. The monkeys having access to each other overnight was probably the most interesting phase of the study (C). Overnight housing placed the monkeys in close quarters and they had the luxury of a bit of privacy. Monkey 1 began to allogroom

Monkey 2 regularly and even more frequently after Phase C. Early in the afternoon on the day following their first night of overnight access, copulation was observed for the first and only time during the entire study. Up until this point the zoo staff was not sure that Monkey 2 was even capable of copulation. I found this phase of the study so intriguing that if I ever get an opportunity to do an introduction study again, I will arrange for night vision cameras to observe the monkeys overnight unobtrusively. The second time Monkey 2 hit Monkey 1 was the day after they were allowed overnight access. This phase also marked an increase in the frequency of touching for Monkey 2.

Phase D, or the bird introduction, had a direct impact on the behavior of Monkey 2. The day the birds were brought into the exhibit marked the third and final time that Monkey 2 hit Monkey 1. The behavior of hitting for Monkey 2 was the most susceptible to experimental treatments. All three occurrences happened after a phase change in the study. Based on this raw evidence, I really thought that of all the behaviors, hitting for Monkey 2 would be the most likely to correlate with higher salivary cortisol levels, but it did not. The bird introduction also marked the peaks for Monkey 2’s movement and climbing frequencies, as well as his propensity to follow after Monkey 1. The presence of the birds disrupted things for Monkey 2 quite a bit. It was shocking when he attacked the first bird and even more surprising when he attacked the second. There is no doubt in my mind that the other birds would have met the same fate had they not been removed from the exhibit. Monkey 1 was used to the birds; she had been sharing an exhibit with them for a long time. When the birds were brought back into the exhibit, for Monkey 1, all was right with the world.

One of the most valuable contributions of this study is solid data on the range of salivary cortisol concentrations for *Alouatta caraya*. To the best of my knowledge none have been reported before. This species of monkey follows the pattern of diurnal variation in cortisol levels that is associated with primates (Kuhar et al. 2005). Cortisol levels are higher in the morning than they are in the afternoons; this was the case for both monkeys. The phases of the introduction also influenced salivary cortisol levels indicating that the presence of a new monkey or a loud, squawking, colorful bird that is foreign to you are significant stressors that can initiate the stress response. The cortisol levels also tell us is that the range found in the howler monkeys is in line with, or at least similar to, those found in other studies on New World monkeys that are of a similar size and weight (Ange-van Heugten et al. 2009). Salivary cortisol levels found in the howlers are much higher than those recorded for their Old World counterparts (Lutz et al. 2000).

As for the relationship between behavior and high salivary cortisol levels (i.e. stress), Pearson's correlations showed a relatively strong relationship for the behaviors of howling and biting in Monkey 2. This relationship is far away from indicating that acts of biting and howling are clear indicators of stress. One of the problems I have with such an interpretation is that the sample size is so small; further investigation is needed to definitively determine this relationship. When the data for the two monkeys is combined, no behaviors show a strong positive linear relationship. I did not include the combined data in the study because of the sex difference between the two animals, especially since males are more likely to be aggressive than females. There also is individual variation in what constitutes a stressor. For example, the two monkeys had extremely different reactions to the presence of the birds because of their previous zoo experiences. It is also common sense when we compare the alternate relationship that individual people have to potential stressors. Once again, what stresses you out might not stress me out, and vice versa.

Cortisol studies in free-ranging populations generally recover cortisol levels from fecal matter. They also tend to measure environmental stressors as did Martinez-Mota et al's. 2007 study which evaluated fecal cortisol in groups living in fragmented versus continuous forests. The literature does not provide comparable values of salivary cortisol in free-ranging populations for *Alouatta caraya*, but based solely on behavioral observations, Monkey 1 and Monkey 2 exhibit typical behavior relative to this species. Among the factors affecting behavior, the largest difference between wild populations and Monkey 1 and Monkey 2 is the size of their range. The captive population can only dwell within the confines of their exhibit. Even so, they can be seen foraging, howling, sunning on their backs, curled up during bad weather, or resting following the same daily patterns as their wild counterparts.

As seen in other investigations, the howler study found salivary cortisol to be a good measure of the physiological response stress. I have identified only one other study that attempts to align certain behaviors with stress, namely Kuhar et al's. 2005 examination of western lowland gorillas at the Cleveland Metroparks Zoo. They were unable to confirm specific types of behavior to be analogous with stress. They were investigating categories of behavior as opposed to the specific behaviors that were monitored for in the howler study. By singling out behaviors, the howler study has indicated that a relationship exists between hitting and howling, and stress.

One of the most significant findings garnered from this study revolves around the changing Phases (A-D) of the introduction. It is zoo policy that the animals be continuously observed on the first day they are introduced. In the event of an unsuccessful introduction, an intervention might be required, so it is necessary to ensure the animals are civil. Therefore, the zoo has a general idea of whether the introduction will be successful or not. Zoo workers look for peaceful cohabitation, time spent huddled together, and lack of aggression as symptoms of a successful introduction. This study indicates that even though the animals appear to get along initially, or that their stress levels returned to normal range immediately after the introduction, that changing conditions can continue to have an effect on stress levels. An example of this is observed in Monkey 2 after Phases C and D. His cortisol levels increased after each treatment. This suggests that the animals are affected by stress due to introduction long after its initiation.

Overall, the design of the study, particularly with the changing phases throughout its duration appeared to be successful. Dividing the introduction into four distinct segments added weight to the salivary cortisol levels, allowing for something to compare them too. We now know that Monkey 1's cortisol was highest the day Monkey 2 was introduced into the exhibit (Phase B); we know that Monkey 2's cortisol levels increased after they were allowed overnight access and again after the birds were introduced (Phases C and D respectively). In hindsight, I should have taken saliva samples from both monkeys every day of the introduction. There was no cortisol level taken for Monkey 2 on the first day of the introduction due to the alternating focal animal design of the study. It would have been possible to observe both monkeys on the same day; it just would have been more difficult. In reality, more researchers would have been required to conduct the study in this manner. It is difficult to keep track of alternating focal animals, and also the focal period sample size could potentially have been compromised if the day was spent alternating focal animals. It would also have been impractical for us to obtain saliva samples from both monkeys each day. Monkey 2 often was not receptive to being swabbed for saliva. Only one zookeeper was free to take the swabs at a time, and trying to swab both of the monkeys would have been too time-consuming.

The largest threat to the validity of conclusions drawn from this study is the small sample size. Evidence for a link between behavior and stress was only found in Monkey 2. Ultimately, both monkeys exhibited typical passive howler behavior. Aggressive acts are most commonly male on male. The best way to define which behaviors constitute stress would be to do a similar study in a group dynamic. Preferably the group would have more than one male and at least one female for whose attention they could compete. This way more animals could be observed and measured, increasing the sample size. I also would have devised a better method of collecting the saliva. The sorbette swabs were effective to collect saliva, but a couple of times the monkey bit off the absorbent tip. Using dental rope as a collection device probably would have provided larger saliva samples; this method was not used because the zoo was concerned about dental rope being a choking hazard.

Zoos generally are public institutions that provide valuable services to the communities they reside in as well as to the well-being of both captive and free-ranging animals. Their purpose is to educate the public about wildlife and also provide a means for conserving it. Many zoos serve as grounds for breeding endangered animals some of which will be successfully reintroduced into the wild. The introduction of unacquainted animals is a common occurrence at zoos and they must be done with care in order to protect the safety of the animals and the integrity of the zoo. This study documented the successful introduction of two unfamiliar black and gold howler monkeys (*Alouatta caraya)*. Behavioral observations allowed for the analysis of each animal's ability to cope with the pressures placed upon them during the introduction. The analysis of salivary cortisol levels allowed for the determination of physiological stress levels. This study provides evidence that howling and hitting behaviors are associated with increased cortisol levels and by extension the physiological stress-response.

Zoos represent only one type of environment that primates live in. Much of primatological research occurs on primates in field locations where they are used to a variety of different levels of human contact, and in laboratories where the relationship between subjects and researchers allow for another level of human contact. Zoo animals are used to multiple levels of human contact such as their relationship to zoo keepers, crowd members and researchers. Behavior should be studied in zoo primates for three basic reasons. Firstly, to ensure their welfare; next, to lead to an understanding of the animals that will facilitate a better experience for zoo visitors; and finally, to ensure that research undertaken by zoos is properly evaluated (Hosey 2005).

Because the physiological stress-response is practically the same throughout the primate order, the implications for anthropology are vast. Primates are studied in anthropology because of their close evolutionary relationship to humans. If we can better understand the stress response in non-human primates and the behavioral signs of stress, then that understanding can be applied to understanding those occurrences in humans.

The study of the behavioral reaction to stress can lead to a reduction in stress related diseases by understanding the causes of the diseases as well as provide insight towards the alleviation of stress. If it is known which behaviors are triggered by the stress-response, then periods of prolonged stress can be reduced by the implementation of stress alleviating activities. The circumvention of prolonged stress will result in a decrease in the onset of stress-related disease which will be beneficial to all walks of humanity.

APPENDIX A

OBSERVATION TABLE

Date: **Time of Observation:**

Weather Conditions: **Location:**

Behavior	**Monkey 1**	**Monkey 2**	**Time Start**	**Time End**	**Monkey 1 Total**	**Monkey 2 Total**
Allogrooming						
Mostly Asleep						
Mostly Awake						
Degree of Movement (1-5)						
Autogrooming						
Body Tremors						
Hitting						
Chasing						
Biting						
Urination/Defecation/Scent Marking						
Supplantation						
Play						
Vulval/scrotal display						
Copulation						
Clitoral display						
Yawning						
Scratching						
Eating						
Climbing						
Howling						
Grunt						
Touching						
Smelling						

APPENDIX B

BEHAVIORAL DESCRIPTIONS

To count as 1 a behavior must occur and stop, a behavior can occur again if it stops completely for 5 seconds. Behaviors with an * were derived from Jones (1983).

Autogrooming – grooming of self

Mostly Asleep **-** more than half of focal period spent sleeping

Mostly Awake – more than half of focal period spent awake

Degree of Movement (1-5) – degree of movement during focal period where 1 is no movement and 5 is constant movement

Allogrooming* – grooming of other

Body Tremors – shivering of the body

Hitting* – intentionally hitting or hitting at another individual

Chasing – following or chasing another individual

Biting* – biting or biting at another individual

Urination/Defecation/Scent Marking – self explanatory

Supplantation * – intentionally displacing other individual to alternate location

Play* – “acrobatic” movements focused around other individual

Vulval display* – presentation of the rear, tail up and body stiff: caution light message; stop or slow down

Copulation* – mounting with thrusting of the pelvis

Clitoral display *– open display of erect clitoris associated with submissive behavior

Yawning – visibly yawning

Scratching – short duration scratching of self

Eating – eating biscuits or foliage

Climbing – movement through exhibit above ground level

Howling – long duration howling or short precursor to howl

Grunt – short vocal grunt

Touching – with fingers or hands

Smelling **-** visibly sniffing other individual, can be associated with play

APPENDIX C

IACUC APPROVAL FORM

The University of Southern Mississippi

Institutional Animal Care and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the three year approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes (see attached) should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: **09051401**
PROJECT TITLE: **The Relationship between Salivary Cortisol Levels and Stressful Behaviors of Black Howler Monkeys (*Alouatta Cayaya*) Upon Introduction at the Hattiesburg Zoo**
PROPOSED PROJECT DATES: **5/11/09-9/01/09**
PROJECT TYPE: **New**
PRINCIPAL INVESTIGATOR(S): **Marie Danforth**
DEPARTMENT: **Anthropology & Sociology**
FUNDING AGENCY/SPONSOR: **Departmental**
IACUC COMMITTEE ACTION: **Full Committee Approval**
PROTOCOL EXPIRATON DATE:

Jodie M. Jawor, Ph.D.
IACUC Chair

11/28/11
Date

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