

An examination of salivary cortisol concentrations and behavior in three captive African  
elephants (*Loxodonta africana*) at Zoo Atlanta

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## SUMMARY

Salivary cortisol is becoming an effective method with which to quantify cortisol levels, including the ability to track diurnal patterns and acute stress fluctuations. The purpose of this study was to validate salivary cortisol for use in African elephants (*Loxodonta Africana*), establish baseline cortisol values in three African elephants at Zoo Atlanta and explore the relationship between cortisol and various behaviors and husbandry events. Elephant salivary cortisol was found to be a valid measure based on correlations with serum cortisol and serial dilution results. Salivary cortisol also decreased across the day, but no definitive patterns were revealed. Using baseline values, salivary cortisol was used to examine the effects of enrichment, maintenance and novel training, and a mild stressor. Maintenance training was found to lead to lower cortisol values than novel training. Salivary cortisol after enrichment did not differ from individual overall means. The mild stressor initiated a rise in salivary cortisol. The final focus of this study was to investigate the link between salivary cortisol and stereotypic behavior. Stereotypies are described as repetitive behaviors with little variance and no discernible function or goal. There is not a straightforward relationship between stereotypies and welfare. Analysis of salivary cortisol at various durations into swaying bouts established that swaying appears to decrease cortisol levels. Additionally, behavioral data were collected. Behavioral data confirmed anecdotal reports of circular dominance in these animals. Behavioral data also revealed that although these individuals spend the majority of their time consuming food, one individual in particular devotes a significant amount of her time to swaying, a percentage much higher than that found when Wilson, Bloomsmith, and Maple (2004) examined stereotypic swaying rates

in these same animals. Results of this study have direct ramifications for the current management requirements for captive elephants around the world. It helps tap into aspects of psychological well being of captive elephants to elucidate factors influencing welfare and stereotypic behavior. Research of this nature is a critical endeavor if we are to appropriately manage these magnificent animals in captivity.

## INTRODUCTION

Since the first African savanna elephant (*Loxodonta africana*) was brought to North America in 1804, they have become popular zoo animals, with approximately 250 individuals currently housed in captivity in North America (Schulte, 2000). However, the keeping of elephants in captivity has become a controversial issue; mainly because of concerns about meeting their welfare needs (see Wemmer and Christen, 2008). Issues of welfare in captive elephants relate to reproduction, food health, obesity, stereotypic behavior, spatial restriction, and lack of exercise opportunities (Clubb and Mason, 2003). The housing of captive elephants often differs greatly from the wild in terms of spatial restriction, physical complexity, and social complexity. It is generally agreed that the captive environment for elephants should be improved, but there is a debate raging on how to do so and little systematic data on which to base decisions. Instead of attempting to replicate nature (Hutchins, 2006), captivity should be evaluated in terms of scientific data on welfare, including both behavioral and physiological measures (Bettinger and Laudenslager, 1998). Elephant welfare, both behaviorally and physiologically, has been examined in remarkably few scientific studies, especially given the controversy surrounding this species and their high level of social complexity, intelligence, and popularity (Brown, Wielebnowski, and Cheeran, 2008). Therefore, this study set out to explore the behavior and salivary cortisol in three captive African elephants as an avenue to explore welfare.

### *Definitions*

Many important terms have no standard scientific definitions, and are generally highly debated, thus their usage must be clarified. The main concepts relevant to this

paper are stress, welfare, well-being, need, want, suffering, frustration, and coping. Occasionally it is even suggested that use of these various terms should be discontinued as valid scientific terms (for discussion, see Ewbank, 1973). However, these terms are important as it would be impossible to discuss cortisol and behavior without them, thus they will be defined for use in this paper.

Stress, according to Friend (1991) is a “complex phenomenon” that has been explored from various approaches and thus “it is unlikely that a single unifying theory will be possible” in the near future (p. 292). In general, hypothalamus-pituitary-adrenal (HPA) activity is one of the most practical metrics of stress (Swaigood, 2007). Stress is often thought of as a negative entity, but stress can be considered an inherent part of being alive (Brown, Wielebnowski, and Cheeran, 2008). Some argue for dividing the term into distress and eustress, but for the purposes of this paper, stress will be defined biologically as activation of the HPA axis (Kirschbaum and Hellhammer, 2000) resulting from physical and psychological demands, or stressors, faced by animals. Additionally, behavioral data will be collected to examine potential links between cortisol and behavior and explore behavioral indicators of stress.

Defining animal welfare or well-being, terms often used interchangeably, is essential for many ethical decisions; however the debate is far from a consensus (Mason and Mendl, 1993). Broom and Johnson (2003) declare that welfare should be viewed as a continuous variable, rather than something that an animal has or does not have.

Definitions of welfare often focus on biological fitness, citing the importance of survival and reproduction. However, others focus on psychological aspects, claiming that welfare can be decreased even without health issues. Many of the definitions of welfare or well-

being include terms, such as needs or wants, which have to be defined themselves.

Dawkins (2004) suggests assessing welfare by examining, through behavior, if animals are healthy and if they have what they want. Answering if they have what they want or need refers to “cases where the animals do not die or suffer ill health if they are deprived, but may nonetheless ‘want’ something in the sense of being highly motivated to obtain it” (p. S3). In these cases, needs can be either physiological, such as those for food or water, or ethological, such as the need of female chickens for dust baths (Dawkins, 1983) or nest building (Hughes, Duncan, and Brown, 1989). Animals are thought to have reduced welfare if they are prevented from performing behavior patterns for which they have a behavioral need to perform (Hughes, Duncan, and Brown, 1989). Others focus the definition of welfare on whether or not a captive animal exhibits the full behavioral repertoire performed by conspecifics residing in the wild (for discussion, see Veasey, Waran, and Young, 1996). Additionally, behaviors exhibited by captive animals but not seen in their wild counterparts, mainly stereotypic behavior, are often speculated to be a principal indicator of poor welfare (Broom and Johnson, 2003). However, captivity should be expected to alter behavior (Veasey, Waran, and Young, 1996) and the lack of the full behavioral repertoire does not equate to undisputable decreased welfare.

Additionally, determining the percentage of behavioral repertoire required to claim that an animal exhibits well-being is a complicated and subjective task (Novak and Suomi, 1988). Hutchins (2006) states that elephant welfare, mainly social needs, can be judged on the basis of exhibiting species-typical social behaviors and low levels of “agitation, aggression, lethargy, or stereotypic movements” (p. 167). According to Mason and Mendl (1993), there is a consensus on measuring welfare through physiological and

behavioral methods; however interpreting these measures is the difficult task. Various measures may lead to contradictory conclusions on the welfare status of an animal, thus it is imperative to use multiple measures when investigating welfare and not focus on a single one. Similar to stress, the definition of welfare, for the purpose of this paper, will focus on physiological and behavioral indices. Welfare will be regarded as an objective quality that can be measured through various indicators (Broom, 2001), mainly non-elevated baseline cortisol and species-typical behavior.

Another term that is relevant to the discussion of welfare is suffering. Suffering can be defined as “an unpleasant subjective feeling that is prolonged or severe” (Brown, Wielebnowski, and Cheeran, 2008; p. 123). Suffering can occur because of physical problems, such as pain, and these measures have established links to welfare (Rushen, 2003) and can elicit a physiological response (Brown, Wielebnowski, and Cheeran, 2008). However, psychological suffering is also possible, when animals are prevented from obtaining something which they are highly motivated to obtain (want or need), thus becoming frustrated. Frustration, or continuing levels of motivation to perform a behavior the animal is unable to perform, is often thought to be indicated behaviorally, potentially through stereotypic behavior. It is speculated that animals often perform displacement behaviors, such as stereotypic behavior, when unable to perform the behavior they are motivated to perform (Mason, 1991; Friend, 1990). For example, carnivores with larger home ranges, motivated to explore, exhibit higher rates of stereotypic behavior in captivity (Clubb and Mason, 2003). However, the link between motivation and stereotypic behavior requires further investigation (Rushen, 2003). Mason and Latham (2004) claim that the majority of research has illustrated that situations that cause or

increase stereotypic behavior also decrease welfare. In contrast they also discuss data that demonstrate that stereotypic behavior does not definitively signify poor welfare. Overall, experiencing suffering or prolonged stress are thought to imperil an animal's welfare. In this study, suffering will be examined through basal cortisol levels and displacement behaviors.

The final relevant term is coping. The term coping is preferred to adaptation given the assorted biological meanings of adaptation (Broom, 2001). Broom and Johnson (2003) discuss coping as a method an animal uses to solve difficulties inherent in its life, including daily regulation of body states as well as responses to emergencies. Jensen (2001) states that an essential feature of coping is deactivation of motivational systems, which were mostly influenced by evolution for existence in the wild because captivity only alters selected behavioral strategies. It is often speculated that stereotypic behavior is an attempt to cope with suboptimal conditions, either current or past. A failure to cope is thought to lead to stress and poor welfare (Jensen, 2001). However, Jensen claims that successful coping is not equivalent to good welfare because the coping may come at such a high cost that welfare is still reduced. For this study, coping will be examined through behavioral means, mainly stereotypic rate and any other potential behavioral adaptations observed, as well as non-elevated baseline cortisol.

### *General Elephant Information*

Wild elephants have sexually dimorphic social behavior. Wild male elephants leave their natal groups after they mature, and occasionally form fluid bull herds (Evans and Harris, 2008; see also Clubb and Mason, 2002; Eltringham, 1982; Laws, 1970). Perhaps because of their extraordinarily long lifespans that create overlapping

generations, female elephants can be said to “have one of the most advanced of all mammalian social systems” (Dublin, 1983, p. 291). Female African elephants in the wild live in close-knit, stable groups that generally consist of related adult females and their young (Charif, Ramay, Langbauer, Payne, Martin, and Brown, 2005). Groups commonly include eight to twelve individuals (Schulte, 2000). Females tend to remain in their natal group, however fusions of unrelated groups have been observed, allowing for the possibility of bonding outside of relatives (Moss, 1988).

Elephant social housing in captivity attempts to imitate wild groups, housing females in groups whenever possible and separating males for single housing (Schulte, 2000). However, Schulte highlights that the adult captive elephant population differs from the wild population because males are removed at a much younger age, groups tend to be smaller, the calf population is extremely low, captive groups tend to consist of unrelated individuals, and individuals are occasionally housed in mixed-species groups with African and Asian elephants (*Elephas maximus*). These differences may be problematic for elephant welfare, especially because species-typical social complexity is essential for many species in captivity (Carlstead and Shepherdson, 2000) and it may be detrimental when group size and complexity fall short of how the species lives in the wild (Veasey, 2006). Nonetheless, strong social bonds appear to be important for even small groups of unrelated captive female elephants and they are reported to display high levels of social behavior (Gruber, Friend, Gardner, Packard, Beaver, and Bushong, 2000; Garai, 1992). In addition, how captive elephants spend the majority of their time may resemble wild elephant behavior, with both spending approximately 75% of their time feeding (wild: Wyatt and Eltringham, 1974; captive: Wilson, Bloomsmith, Crane, and Maple,

2001). However, some studies report time captive elephants spent foraging and drinking to be as low as 25% (see Clubb and Mason, 2002). Additionally, food for captive elephants is “more spatially and temporally concentrated and contain[s] much less variety” (Stoinski, Daniel, Liu, and Maple, 2000, p. 485-6). Elephants in the wild are known to be generalist herbivores and are reported to eat hundreds of different species of plants, not just hay and a few browse species (Hancocks, 2008; Sukumar, 2008). This reduction in variation can lead to boredom and the captive diet may also be a large factor in the obesity seen in many captive elephants (Hancocks, 2008).

An additional difference between life in the wild and in captivity are spatial restriction. Elephant feeding behavior in the wild tends to include some locomotor aspects, with feeding at each spot generally limited to only a few minutes (Wyatt and Eltringham, 1974; McKay, 1973). Thus, wild elephants can cover quite a bit of ground while feeding, suggesting that they have large home ranges. The exact effects of spatial restrictions seen in captivity on elephants are unspecified, but large home ranges have been correlated with stereotypy performance in carnivores (Clubb and Mason, 2003). Although it is unlikely that zoo elephant exhibits could ever be as spacious as the home ranges of wild elephants, not many current exhibits even meet the Association of Zoos and Aquariums (AZA, formerly American Zoo and Aquarium Association) current standards of at least 1.5 acres (6070.5 square meters) per elephant (AZA, 2003). According to a survey by Bashaw, Burks, Daniel, and Maple (1999), many elephants are confined to indoor areas at night and the average elephant has only about 183 square meters of indoor space. Elephants are also often confined to smaller indoor enclosures during colder weather. The amount of time spent in smaller areas is potentially

problematic because Elzanowski and Sergiel (2006) found that an elephant who spent 52% of her time stereotypically swaying spent significantly less time swaying when she was regularly allowed into her 650 square meter paddock than when releases into her paddock were irregular and she was mostly confined in an indoor pen of 30 square meters. Therefore, even though quality of space has been deemed more important than quantity for some species (e.g. Perkins, 1992), such large animals warrant large enclosures and may experience decreased welfare when space is limited.

An even greater restriction of space occurs when circus elephants and those housed in zoos have had their legs chained, greatly restricting possible movement and performance of species-typical behaviors, such as foraging, social behavior, and locomotion. Attempting to cope with this restriction may lead to the stereotypical behavior seen in captive elephants (Gruber et al., 2000). Chaining provides harsh restrictions on movements, leaving only enough space for movement approximately one meter forward or backward and almost no sideways movement. The practice of chaining has decreased based on careful observations of unchained Asian (Wiedenmayer and Tanner, 1995) and African elephants (Brockett, Stoinski, Black, Markowitz, and Maple, 1999). The studies determined that unchained elephants did not exhibit high rates of aggression and actually exhibited decreased stereotypic behavior. However, chaining is still used by some institutions today. According to a survey by Bashaw, Burks, Daniel, and Maple (1999), 28% of elephants housed in AZA accredited zoos are restricted by leg chains for some portion of the night and 20% are not allowed access to conspecifics overnight. Stereotypic behavior has been shown to occur at significantly higher rates in

chained than penned circus elephants (Gruber, et al., 2000; Friend and Parker, 1999). Thus the practice of chaining is likely detrimental to elephant welfare.

### *General Stereotypy Information*

Stereotypies are generally defined as repetitive behaviors with little variance and no discernible function or goal (Ödberg, 1978). These behaviors have long been thought to reflect past exposure to sub-optimal environments and decreased welfare (e.g. Hediger, 1964). Stereotypies often develop in animals in restricted environments, such as those seen in captivity. In some cases, spatial restriction has not greatly affected abnormal behavior rates or urinary cortisol levels (see Crockett, 1988). Draper and Bernstein (1963) did find increased rates of stereotypy in rhesus monkeys with increased space restriction. This same relationship has been seen in giant pandas (Liu, Wang, Tian, Yu, Zhang, Wei, and Zhang, 2003) and felids (Moreira, Brown, Moraes, Swanson, and Monteiro-Filho, 2007; Mallapur and Chellam, 2002). Stereotypy rates in elephants are increased when animals are chained or picketed versus penned (Gruber, Friend, Gardner, Packard, Beaver, and Bushong, 2000; Friend and Parker, 1999), an extreme version of spatial restriction. The majority of the data support the conclusion that stereotypic rate increases with spatial restriction, however, space is not always the critical variable.

Forsyth, Young and Mench (2007) found that spatial complexity, as measured by level of enrichment, was a more important determiner of stereotypic level in female mice housed in cages. Therefore, environments should be improved both in terms of quantity and quality of space, but in some situations quality is the only aspect that can be modified.

Evidence suggests that many current exhibits are still inadequate, and the restricted space

and poor enclosures still trigger abnormal behavior, despite the enormous improvements over the desolate cages of the past (Lyons, Young, and Deag, 1997).

Even beyond spatial restriction, life in captivity is far removed from the environment for which the animal has adapted through natural selection (Mason, 1991b). According to Hughes and Duncan (1988), an animal in captivity goes from deciding how to allocate limited time among behaviors crucial to survive to how to spend its abundant time available within the behavioral constraints applied by captivity. Wooster (1997) states that because the captive environment satisfies the animal's biological needs, the motivation for exploring the environment and utilizing survival skills and sensory abilities is reduced, leaving a potentially bored and inactive animal. Certain stereotypies, including pacing, resemble forms of normal behavior (Mason, 1991a) that animals may be motivated to perform, suggesting a need to consistently include enrichment and training in captive settings to provide animals an option for spending their excess time.

Stereotypies may vary in form by species, and ultimate explanations based on ecological niche and evolution can help elucidate these differences in reactions to captivity (Clubb and Mason, 2004). An informative study by Clubb and Mason (2007) examined the tendency for stereotypic development in carnivore species that differed in behavior in the wild (see also Clubb and Mason, 2003). They correlated median stereotypic levels with the main available explanatory hypotheses. Despite widespread support for the hypothesis that stereotypic levels are related to natural foraging or hunting specialization or activity levels, it was found that stereotypic levels were explained by differences in natural ranging behavior, mainly territory size and median daily travel distance. Stereotypic behavior often develops in animals that are innately active or those

that patrol a territory in the wild (Weschler, 1991). Although these analyses examined carnivore species, their findings may transfer to elephants with large home ranges of up to thousands of square kilometers and daily travels of several kilometers (Clubb and Mason, 2002).

In terms of captive elephants, the main stereotypic behavior is a repetitive sideways body movement in which the elephant generally remains in place, although it may lift one or more feet. These movements may involve head swaying and swinging movements of the trunk and is referred to as swaying or weaving. This stereotypy was seen in a portion of captive elephants that were kept under intensive housing systems, mainly chaining (Kurt and Garai, 2001).

#### *Stereotypy and Welfare*

There is not a straightforward relationship between stereotypy and welfare and it does not appear that greater stereotypy rates always indicate worse welfare (Mason, 1991b). Stereotypy rates vary greatly in captive animals. For instance, in one study of three polar bears, Weschler (1991) found that 16.0%, 24.4%, and 76.5% of the observation time was devoted to stereotypic behavior. It has been suggested that if stereotypies occupy more than 10% of an animal's activity budget or causes bodily injury, it is probably detrimental to welfare (Broom, 1983). However, there are problems in creating an arbitrary cut-off to use to judge welfare.

Additionally, stereotypies frequently become independent of the initial eliciting situation, which obscures the connection between stereotypy performance and decreased welfare. Animals that perform stereotypies should be considered at risk for welfare decrements (Swaigood and Shepherdson, 2005), mainly because of the consequences

associated with stereotypic behavior. They tend to exhibit less behavioral diversity (Dantzer, 1986) and often lose portions of species typical behavior patterns (Shyne, 2006), suggesting to some that stereotypic behavior is proof of diminished welfare and suffering (Broom, 1983).

Stereotypies should be taken seriously, but not used as definitive indicators of poor welfare (Mason and Latham, 2004), because although they may develop in sub-optimal environments, after establishment, they will be performed even after the environment is improved. For example, transferring bank voles from a barren environment in which they establish stereotypes to an enriched cage does not necessarily lead to decreased stereotypy rates (Cooper and Ödberg, 1991). Therefore, the development of stereotypies may signify a suboptimal environment, but performance of established stereotypies does not equate to current welfare detriments, although it may imply current suffering (Mason, 1991b). Because of the complexity of this interaction, it is essential to examine the former and current effects of stereotypic behavior.

Once established, stereotypies are generally habitual and difficult to disrupt and are performed even though they carry energy costs, suggesting that they may be reinforcing to the animal (Mason, 1991a) or fulfill a need (Ödberg, 1986), thus having some level of function. The most substantial evidence that stereotypies may be reinforcing is that animals will work to be allowed to perform them, such as a dominant animal displacing a subordinate to gain access to a preferred stereotypy location (Mason, 1991a). Potential reinforcement and independence from developing stimulus can make stereotypies difficult to decrease once they develop (Mason, 1991a). In addition, they can be affected by many factors, such as enclosure size (e.g. Lyons, Young and Deag, 1997)

and feeding regime (e.g. Jenny and Schmid, 2002; Carlstead, Seidensticker and Baldwin, 1991), making fundamental causal factors hard to isolate, especially in studies with only a few subjects.

### *Stereotypy and Age*

Vickery and Mason (2004) state that animal caretakers often rush into attempts to lessen stereotypic behavior and fail to take the time to understand the behavior.

According to Kiley-Worthington (1990), it may not be possible to eliminate stereotypic behavior in adult animals, only reduce their dependence on such behaviors. However, with a better understanding of stereotypic behavior development, it should be possible to design optimal environments that reduce the development of stereotypies in young animals.

There are often differences between juvenile and adult animals that perform stereotypic behavior. For example, young rats beginning to perform stereotypic backflips exhibit highly variable locomotion as the stereotypy develops, occasionally including an extra wall or adding a jump (Callard, Bursten, and Price, 2000). Young mink, in comparison with adults have lower rates of stereotypic behavior and do not exhibit the same level of invariant behavior (Mason, 1993). This distinction was also seen in captive brown bears (*Ursus arctos*, Montaudouin and Le Pape, 2005). Young brown bears exhibited less and a different form of stereotypic behavior than adult bears.

Additionally, stereotypies often develop over greater time periods as an animal attempts to cope with an environmental situation and behavior becomes more restricted, repetitive, and invariant (Dantzer, 1986). Kurt and Garai (2001) traced the development of swaying in young captive Asian elephants in Sri Lanka. They found that movements

which began as complete steps and exploratory trunk movements were reduced to swaying with increasing age and time in captivity. This restriction progressed to the point that after only 3 months in captivity, young elephants were devoting more than 50% of their time swaying. Additionally, the frequency of the swaying behavior slowed from 30 movements per minute in younger animals to only ten movements per minute in older animals. Overall, the development of stereotypic swaying in elephants (Kurt and Hartl, 1995) resembles that seen in tethered female pigs (Cronin, Wiepkema, and Hofstede, 1984), which includes an initial stage of anxious resistance followed by a period of drowsiness which changes to a stage of repetitive exploratory behavior that shifts to stereotypic behavior (Kurt and Hartl, 1995) as the behavior becomes less intensive, repetitive, and then ritualized (Wiepkema and Schouten, 1992).

Age may affect stereotypic behavior in elephants, with younger elephants sometimes exhibiting more stereotypic behavior (Gruber, et al., 2000). However, Wilson, Bashaw, Fountain, Kieschick, and Maple (2006) observed stereotypic behaviors not seen in the same elephants when observed overnight by Brockett, et al. (1999). Although, the percentage of the activity budget devoted to stereotypic behavior was small, only 3.5% of scans for stereotypic behavior and 2.7% was swaying. Additionally, Wilson et al. (2006) began observations an hour earlier, 1700 versus 1800 in Brockett et al. This additional hour covered the shifting of the animals into the barn and leaving of the handlers. Although swaying did peak during this hour, an additional peak was seen in the morning corresponding to the arrival of the handlers, approximately 0800, the time at which both studies ended data collection. Perhaps these methodological differences account for apparent age differences in swaying. Therefore, it is unclear how age affects stereotypic

performance in elephants. Overall, stereotypic behavior in elephants, especially its function, needs to be further examined.

### *Stereotypy Causes*

Stereotypies generally develop in situations of frustration, unavoidable stress or fear, restraint, and lack of stimulation; all situations of decreased well-being (Mason, 1991a). Many risk factors for abnormal behavior have been identified for non-human primates. In one study examining several non-human primates, several key factors were identified that influence abnormal behavior (Mallapur, 2005). These factors were group composition and size, enclosure design, and rearing history. Additional studies have highlighted the significance of an impoverished early environment (Lutz, Well, and Novak, 2003; Bellanca and Crockett, 2000; Harlow and Harlow, 1962), but causal factors vary by species. For example, it appears that early rearing does not affect avian stereotypies (Keiper, 1978).

In examining how a stereotypy may develop, performing certain behaviors may be reinforcing for the animal and this reinforcement leads to a behavioral need so that prevention of performing the behavior may decrease welfare, even if the behavior is unnecessary in the captive situation (Wiedenmayer, 1997; Hughes, Duncan, and Brown, 1989). Situations in which an animal is prevented from performing a behavior, such as searching for food or mates or escaping from an enclosure, can lead to movement stereotypies. For example, stereotypies developed from escape movements displayed by hungry female chickens that could see but not acquire food in just a few sessions (Duncan and Wood-Gush, 1972). In addition, delaying feeding in stump-tailed macaques

was found to lead to an increase in abnormal behavior, perhaps because of increased frustration over feeding becoming unpredictable (Waitt and Buchanan-Smith, 2001).

According to Ödberg (1978), excessive arousal can lead to stereotypies in cases of unavoidable stress or fear, or even frustration that functions to produce physiological arousal equivalent to fear. These stereotypies often develop in times of increased stress, such as initial tethering in pigs (Wiepkema and Schouten, 1992) or artificial weaning in rats (Callard, Bursten, and Price, 2000), suggesting that stereotypic behaviors may lower arousal. Stereotypies have been shown to reduce arousal and signs of stress in correlational studies (Mason, 1991a and 1991b). Support for this claim comes from studies which have linked stereotypic behavior and cortisol. For example, prevention of jumping stereotypies in bank voles leads to increased corticosteroid levels (Kennes, Ödberg, Bouquet and De Rycke, 1988). Battery hens have been found to have normal cortisol levels once they become accustomed to their cages and stereotypic behavior becomes habitual (Beuving, 1980). Further support comes from behavioral studies. Restricted space led circus tigers to pace throughout transport if they had not been allowed to exercise before transport (Nevill and Friend, 2003). However, those allowed to exercise and perform pre-transport rested during transport and only paced near the end of the journey. These results suggested that allowing pre-transport exercise decreased the need to decrease arousal during the trip, allowing the animals to rest during the trip.

A contradictory explanation of stereotypies is that they are associated with cases of understimulation, mainly restricted or barren environments, perhaps because of a need for sensory stimulation or a need to attain optimal arousal levels (Mason, 1991; Broom, 1983; Berkson, 1967). Bassett and Buchanan-Smith (2007) claim that captive animals

have severely reduced control, especially in terms of altering the amount of stimulation they receive. Wild animals are able to engage in behaviors, such as approaching or hiding from a stimulus, to control the amount of stimulation they receive and alter it to an acceptable level. They can reliably decrease motivations by engaging in appropriate behaviors, such as searching for food or mates, options that are often limited for captive animals. One way for captive animals to cope with boredom is to engage in appetitive behavior that may develop into a stereotypy to which the animal resorts when it is motivated to perform a consummate behavior (Weschler, 1991). Overall, stereotypies may be coping responses to the eliciting environment that improve well-being, perhaps through the endorphin system.

One essential question to help differentiate between the two possibilities is whether animals are less attentive to the environment or have an uninterested posture during stereotypy performance (Wiepkema, 1982). Ödberg (1978) claims that decreased reactivity to the environment occurs during stereotypy performance. Behavioral data can help elucidate how stereotypies affect arousal levels in many species. Weschler (1991) examined the spatial and temporal patterns of stereotypic walking in polar bears. He hypothesized that polar bears begin their stereotypic walking when they exceed a certain arousal level to decrease arousal. By examining pre- and post-stereotypic behavior and changes in the speed during stereotyped walking, it is suggested that stereotypic walking may reduce arousal in at least one bear. However, the results are not conclusive because there was no difference in stereotypic walking duration when a bear was resting before stereotypic behavior, and thus should be less aroused, or engaging in variable walking or swimming, activities suggesting a higher arousal level. In addition,

the pattern of higher activity levels before and lower activities after stereotypic walking, which would have suggested higher arousal levels before and lower after, was not found. Another finding of interest from this study was that the polar bears were not inattentive during stereotypic walking; a finding that inspired another study to test attentiveness to novel stimuli in pacing polar bears (Weschler, 1992). In that study he placed spots of novel scents in the paths in which polar bears tended to pace. He found that the polar bears performed more sniffing behavior than polar bears pacing on a path without scents marks. In addition, the polar bears would frequently pause pacing to look up. These results suggest that pacing polar bears are not inattentive to their environment during the pacing and that stereotypic pacing may have developed from appetitive behavior.

Feeding procedure in captive animals generally includes processed food which requires only a small proportion of the amount of time allocated to acquisition and consumption of food in the wild, which research suggests does not meet their physical or psychological welfare needs. This deficit has been well studied in carnivores (Law, 1991; Bond and Lindburgh, 1990). Mellen (1991) suggests examining this diet for not only its nutritional components, but also for its impact on behavior. Given that elephants are also fed more concentrated and less varied food in captivity than they would consume in the wild (Stoinski, Daniel, Liu, and Maple, 2000), they too may have welfare deficits based on the typical modes of feeding in captivity that may lead to stereotypic behavior.

Stereotypic behavior in many animals occurs immediately before feeding times. Waitt and Buchanan-Smith (2001) suggest that this behavioral response may develop because the animal has no control over the timing on these events. They state that, given the lack of control over feeding, which is generally highly stimulating for captive

animals, schedules should remain predictable. Predictability allows animals to determine periods in which the event is unlikely to occur, and even small delays may exacerbate the stereotypic behavior patterns seen during the pre-feeding period. Indeed, Waitt and Buchanan-Smith found that delaying feeding led to an increase in inactivity, vocalizations, self-directed behavior, and abnormal behavior in stump-tailed macaques. However, animals have adapted to an unpredictable environment in the wild and too much predictability in captivity may cause boredom induced stress (van Rooijen, 1991). In fact, other studies have found that a highly unpredictable schedule leads to less inactivity and abnormal behavior in chimpanzees (Bloomsmith and Lambeth, 1995). The crucial aspect to the link between predictability and stereotypic behavior may be the pre-feeding cues that induce stereotypic behavior by signaling imminent feeding. Fully unpredictable feeding may prevent animals from learning cues.

This pattern of pre-feeding movement stereotypy has been seen in many captive animals. This effect has been well studied in captive felids (Moreira, Brown, Moraes, Swanson, and Monteiro-Filho, 2007; Weller and Bennett, 2001; Lyons, Young, and Deag, 1997), with many species pacing more in the hour before feeding, suggesting that stereotypic behavior is related to feeding routine. Moutaudouin and Le Pape (2004) found that brown bears (*Ursus arctos arctos*) paced more intensely immediately before feeding time and they would often glance towards cage doors or keeper paths, suggesting the stereotypy may be linked to food expectation. A study by Jenny and Schmid (2002) also found a decrease in stereotypic pacing in Amur tigers (*Panthera tigris altaica*) when they introduced electrically controlled feeding boxes. However, the stereotypic behavior of two snow leopards (*Uncia uncia*) was unaffected by the addition of feeding boxes.

Therefore, the effectiveness of changing feeding routine may depend on the method that feeding animals use in the wild.

This pre-feeding stereotypic behavior pattern has also been observed in elephants. Elzanowski and Sergiel (2006) performed a study on a single elephant that swayed an average of 52% of the time she was observed. When indoors, her swaying was spread mainly between 1000 and 1300. However, when she was let outdoors occasionally, she swayed more in the hour before the feeding time on the indoor schedule and when she was outdoors regularly, she swayed more in the hour before she was let in to be fed. . Additionally, Wilson, Bloomsmith, and Maple (2004) found that the same elephants used in the present study exhibited stereotypic swaying more frequently before the afternoon shifting into the barn than before the morning shift time. The afternoon shift time resulted in access to the evening meal, thus these elephants were displaying pre-meal stereotypic behavior despite the fact that they are provided with food throughout the day. Therefore, these animals, especially one elephant that swayed for a mean of 66% of the 15 minute afternoon shift sessions, may be stressed by predictable husbandry events such as shifting. A similar afternoon swaying peak was found in Asian elephants, with stereotypic swaying rates increasing across the day from 1000 to 1400 hours, when the elephants were shifted into their indoor enclosure and provided with their main meal (Rees, 2004). These findings suggest that elephant stereotypies may be linked to frustrated appetitive behavior. However, circus elephants also show an anticipatory increase in swaying before feeding, cleaning, water provisioning, and even before rehearsals and shows (Kurt and Garai, 2001), so perhaps it is anticipation of a wider

variety of scheduled events. Therefore, stereotypic behavior performance may be related to high levels of arousal.

Although feeding frustration can be linked to many forms of stereotypies, there are also many other potential stressors in captivity that can cause stereotypic behavior. One of the major stressors discussed by Carlstead and Shepherdson (2000) is the inability to escape or avoid aversive stimuli because of their restricted space. Dogs subjected to solitary, restricted housing, were more excitable and exhibited more repetitive behavior (Beerda, Schilder, van Hooff, de Vries and Mol, 1999). Other stressors in captivity include zoo visitors, invasive procedures, isolation of social species and excessive noise. For example, fennec foxes (*Fennecus zerda*) exhibited higher rates of stereotypic pacing during and after machine noises, such as those of a vacuum cleaner or lawn mower (Carlstead, 1991). Days classified as having louder noise were also associated with agitated behaviors; mainly increased locomotion, vocalizations, scratching and door directed behavior; and increased urinary cortisol in giant pandas (Owen, Swaisgood, Czekala, Steinman, and Lindburg, 2004). A potential stressor relevant to the current study animals is the isolation of social species. These elephants occasionally have to be isolated, including overnight, for various reasons, including prevention of aggression. It is unclear whether the isolation or potential aggression is more stressful for these animals.

#### *Effects of Stereotypic Behavior*

Often the effects of stereotypic behavior are unknown. The behavior may aid in coping currently, may have aided in coping previously and continues because it is an established behavior, or it may have always been an indicator of a behavioral pathology (Broom and Johnson, 2003). Friend (1991) suggests that stereotypic behavior aids

coping with environments that are lacking stimulation. If stereotypies do indeed aid in coping, they serve a proximate function, thus challenging the view that they are pathological, functionless behavior (Würbel, Freire, and Nicol, 1998). Wiepkema, van Hellemond, Roessingh, and Romberg (1987) found that in a study of twenty-seven veal calves, the third that developed a tongue playing stereotypy did not develop abomasal ulcers, whereas those who did not engage in stereotypic behavior developed abomasal ulcers or scars, suggesting the stereotypic behavior had a coping effect. Cooper and Nicol (1991) found that bank voles with established stereotypies displayed less preference for enriched environments over barren ones, suggesting that the stereotypic behavior allows the voles to cope with the aversive conditions, potentially lowering environmental awareness. However, Friend (1991) points out that these coping behaviors can result in negative consequences, such as foot injuries to pacing animals and premature damage to the teeth of horses that engage in cribbing behaviors. Thus, these behaviors, even if they allow for coping, may lead to decreases in welfare measured through other means, such as health.

#### *Enrichment and Stereotypic Behavior*

The use of environmental enrichment has grown in the last few decades to improve the captive environment (Chamove and Anderson, 1989) and to help meet the psychological needs (Carlstead and Shepherdson, 2000) and biological functioning (Newberry, 1995) of captive animals. Captive environments present a huge challenge for captive animals and environmental enrichment and training can furnish behavioral alternatives through which animals can respond to their environment (Seidensticker and Doherty, 1996; Carlstead and Shepherdson, 1994). Environmental enrichment is the

most frequently used technique to deal with suboptimal welfare (Swaisgood, 2007), increase behavioral diversity (Chamove, 1989), aid in the ability to cope with challenges (Chamove and Moodie, 1991), and decrease abnormal repetitive behaviors (Mason, Clubb, Latham, and Vickery, 2007) and aggression (Markowitz, 1982). A meta-analysis of 54 studies of the effects of enrichment on stereotypic behavior concluded that enrichment is actually an effective tool for reducing stereotypic behavior but not eliminating it completely (Shyne, 2006).

Environmental enrichment is anything used to improve psychological and physiological welfare of animals in captivity (Shepherdson, 1998), and as such is for all practical purposes a catchall term (Swaisgood and Shepherdson, 2005), meaning that it can include improvements ranging from objects to social companions. Although this definition usually refers to devices and objects placed into an exhibit, training can also be used as a form of enrichment to help decrease stereotypic behavior and increase welfare (Laule and Desmond, 1998; Laule, 1993). Additionally, enrichment could potentially include events that may induce arousal, such as brief threatening events, assuming they are determined to be enriching and not detrimental to welfare (Moodle and Chamove, 1990). Thus, there are numerous options to use as enrichment for captive animals. If implemented correctly, enrichment can address the underlying problems leading to abnormal behavior and stress reactions and if they fail; they can at least provide additional behavioral opportunities and altering the animal's activity budget (Mason et al., 2007). The effectiveness of enrichment items can be examined through usage time, stress hormone levels, and behavioral change, notably increases in stereotypic or other abnormal behaviors and increases in species-typical behaviors. Even if enrichment does

not reduce stereotypic behavior rates, its provisioning may be indicated by the frequency of its use (Crockett, 1988).

Although stereotypies are occasionally performed even after the environment is improved, there are some cases in which environmental improvement or enrichment has been able to decrease the percentage of time spent in stereotypic behavior. Providing a synthetic fleece pad to individually housed laboratory cynomolgus monkeys (*Macaca fascicularis*) allowed tactile stimulation and led to a 53% reduction of cage stereotypic behavior, with further reduction with the addition of foraging crumbles (Lam, Rupniak, and Iversen, 1991). Other primate studies have also found that enrichment leads to a decrease in abnormal behavior (e.g. Baker, 1997; Bayne, Dexter, Campbell, Yamada, and Suomi, 1991; Bloomsmith, Alford, and Maple, 1988; Brent and Belik, 1996). Additional studies have also found increases in species-typical behaviors and a decrease in stereotypic behavior in felids (Wooster, 1997; Markowitz, Aday, and Gavazzi, 1995; Shepherdson, Carlstead, Mellen, and Seidensticker, 1993; Bashaw, Bloomsmith, Maple, and Marr, 2000). Changing circus elephant husbandry procedures so that they were penned instead of picketed significantly decreased stereotypic swaying, head bobbing, and trunk tossing (Friend and Parker, 1999; Friend, 1999).

Not all enrichment attempts may actually improve the environment for the animal (Wiedenmayer, 1998; Priest, 1993). Some individuals may find novelty or unpredictability stressful (Mason, Clubb, Latham, and Vickery, 2007) and therefore not enriching. Additionally, enrichment should be monitored for extinction and habituation (see Tarou and Bashaw, 2007; Carlstead, Seidensticker, and Baldwin, 1991). Enrichment items can be costly; both in terms of cost of the item and personnel time, and can be

potentially harmful (Crockett, 1998). Thus, as much as feasible, each form of enrichment should be tested and not simply assumed to work.

All enrichment items are not created equal. For instance, hiding food in manipulatable objects reduced stereotypic pacing of American black bears from a median of 150 minutes per day to only 20 minutes. However a mechanical feeding device did not significantly reduce pacing (Carlstead, Seidensticker, and Baldwin, 1991). Feeding enrichment was found to have no effects on stereotypic behavior or fecal cortisol in four adult giant pandas (Liu et al., 2006). Occasionally enrichment can actually increase stereotypic behavior, as seen when Fennec foxes were provided with a sand substrate (Carlstead, 1991). Enrichment types must be appropriate for the species in order to perform as intended. Determining appropriateness requires careful study because high quality published research on the effectiveness of enrichment is lacking in many species, especially those outside of the charismatic megavertebrates (Swaisgood and Shepherdson, 2005).

An additional issue with using enrichment to decrease stereotypic behavior is that stereotypic behavior is sometimes harder to reduce via environmental enrichment in older animals, as seen in bank voles that were transferred from conditions that repeatedly lead to stereotypic behavior in young animals to one that rarely did (Cooper, Ödberg, and Nicol, 1996). However, Renner and Lussier (2002) found that novel enrichment objects and a climbing structure reduced stereotypic pacing and increased behavioral diversity and enclosure usage in a 19 year old male and 12 year old female spectacle bear (*Tremarctos ornatus*). Additionally, provisioning novel enrichment to captive adult harbor seals (*Phoca vitulina concolor*) and grey seals (*Halichoerus grypus*), including

some that were over 25 years old, resulted in decreased stereotypic behavior, which was pattern swimming, and increased exploratory behavior and general activity levels (Hunter, Bay, Martin, and Hatfield, 2002).

Additionally, stereotypic behavior may be decreased during enrichment with no delayed effects, suggesting that changing feeding regime alone may not be enough. Feeding enrichment in spectacled bears (*Tremarctos ornatus*) increased foraging behavior when the enrichment was available, but there were no lingering decreases in stereotypic behavior (Fischbacher and Schmid, 1999). Similar results were found in Asian elephants (*Elephas maximus*). Wiedenmayer (1998) suggests that elephants in captivity do not spend enough time foraging, but peanuts hidden in exhibit stone borders did not increase searching behaviors. Perhaps hiding only 25 peanuts was not enough to function as enrichment, thus additional work is needed. Overall, the same elephants that participated in the current study were found to forage similar amounts as wild elephants (Wilson, Bloomsmith, Crane, and Maple, 2001). However, foraging in captivity is not as complex as in the wild, based on the social and spatial restrictions. Additionally, captive elephants spend little time locomoting, unlike their wild counterparts. Therefore, it appears that captive elephants could greatly benefit from enrichment (Shepherdson, 1999). Several authors discuss ways to enrich elephants (e.g., Mellen, Barber, and Miller, 2008; Green, 1993; Schmidt, 1973), including Bashaw, Burks, Daniel, and Maple (1999), that reports that all of the institutions that responded to their survey provided browse as a form of enrichment. However, systematic data are lacking on how enrichment affects elephant behavior (Clubb and Mason, 2002). Morimura and Ueno (1999) found that providing elephants with an additional feeding, two instead of one, actually resulted in

more stereotypic behavior and less time spent feeding. The same methodology had the opposite effect on bears and chimpanzees. Therefore, elephant feeding may be more complex than that of other animals, perhaps because of their substantial food needs. Possibly, elephants actually require more complex feeding methods. A study by Stoinski, Daniel, Liu, and Maple (2000) examined how substituting browse for a portion of their hay affected elephant behavior. They found that in the time subsequent to feedings during baseline hay presentations, the elephants spent 47.0% (pre-treatment) and 51.5% (post-treatment) of their time consuming hay. This percentage increased to 80.0% after browse presentation. Browse is more complicated to eat, and therefore required longer handling time. The increase in feeding was also seen in afternoon sessions in which browse was never presented. However, although the time spent feeding increased from 20.5% during the pre-treatment baseline to 36.7% during treatment, it remained higher at 33.5% in the post-treatment baseline. Also, there were no differences in social or abnormal behaviors, specifically feces manipulation. Additional research on how food enrichment affects captive elephants is essential, especially given the tendency for captive elephants to become obese which limits the recommended amount of high energy foods, such as fruit and grain (Hatt and Clauss, 2006), which are often used as enrichment items for scatter feeding or feeders. Overall, the relation between enrichment and stereotypic behavior in elephants needs to be further examined.

Training can be viewed as a form of environmental enrichment (Laule and Desmond, 1998; Laule, 1993), and is frequently done with elephants. In fact, the American Zoological and Aquarium Society currently requires all elephants to be trained for husbandry and veterinary procedures (AZA, 2003). According to Martin (1999)

positive reinforcement training allows animals some degree of control and choice, which can increase the stimulation of the captive environment. It has been demonstrated that laboratory pigeons (Neuringer, 1969), starlings (Inglis and Ferguson, 1985), rats and various primates (see Inglis, Forkman, and Lazarus, 1997) will work for food, even when the identical food item is freely available. The findings highlight the value of training. Of the 60 institutions that responded to the survey by Bashaw, Burks, Daniel, and Maple (1999), 97% reported that their elephants received some form of training, averaging two hours of training related activities per day. In terms of welfare, training can help increase activity levels and challenge these magnificent animals to aid in coping with the deficiencies of captivity, mainly restricted space for exercise and inadequate opportunities to meet the psychological needs of elephants (Roocroft and Zoll, 1994).

One aspect of elephant training that has been examined and highly debated is the difference between free and protected contact. Protected contact is a management style which, as the name implies, requires all people interacting with the animal to do so through a protective barrier. This method is the standard for most potentially dangerous zoo animals, but is different from the free contact in which elephant keepers become a part of the herd and are actually able to share the same space, thus allowing them to have full access to the elephant. Protected contact was first reported as an experiment used at San Diego's Wild Animal Park to deal with their potentially dangerous bull elephants (Priest, 1992). More and more zoos, especially in North America, are phasing out free contact (see Clubb and Mason, 2002), partially because of the greater risk to handlers seen in free contact (Gore, Hutchins, and Ray, 2006). Free contact often involves physical punishment and requires the humans to force compliance and maintain

dominance over elephants. Protected contact does not require dominance and compliance is voluntary, thus training is accomplished through positive reinforcement methods. Thus, training is vastly different under these two management styles. It is thought that protected contact allows the elephants to derive greater benefits from training because they are not forced to comply. Additionally, all of the standard behaviors required for elephant husbandry, such as foot care and bathing, are possible in a protected contact system. The largest potential problem for protected contact systems is that the transition, especially the removal of the handler from the social group, may be stressful to the animals. The elephants used in the current study are housed under protected contact, thus it is crucial to examine how they are affected by training.

Considering the difficulties of assessing issues such as the potential benefits and deficiencies of enrichment and the possibility of a function of stereotypic behavior, an objective method could prove to be extremely advantageous. The goal of this objective method would be to establish a basis of comparison that could begin to integrate animal welfare, environmental enrichment, behavior, and physiology. The methodology used to instigate this possibility in this work was a combination of behavior and salivary cortisol.

#### *General Cortisol Information*

The various physical and psychological demands faced by animals, labeled stress, lead to a rapid and specific reaction of the hypothalamus-pituitary-adrenal (HPA) axis (Kirschbaum and Hellhammer, 2000). This reaction results in cortisol, a hormone produced by the HPA axis, being regarded as a physiological marker of well-being (Bettinger and Laudenslager, 1998). This response activates mechanisms that help the animal survive during stressful situations by mobilizing energy needed (Möstl and Palme,

2002). Although the HPA axis response includes other hormones, the main stress hormone is cortisol (Kirschbaum and Hellhammer, 1989). Cortisol is a key component to the flight or fight response (Bettinger and Laudenslager, 1998) and has been shown to increase memory functioning in caching birds (Saldanha, Schlinger, and Clayton, 2000).

Cortisol is released into the blood from the adrenal glands. A small portion, about 2-15%, of the cortisol remains unbound or “free” in the blood, while the remaining cortisol binds to carriers such as corticosteroid-binding globulin (CBG), albumin, and erythrocytes (Kirschbaum and Hellhammer, 2000; Beerda, Schilder, Janssen, and Mol, 1996). A radioimmunoassay (RIA) procedure in which the ability of a nonradiolabeled antigen to bind to specific antibody molecules is compared in a standard and the unknown solution is generally used to assess cortisol levels (Reimers, Cowan, Davidson, and Colby, 1981). In addition, cortisol can be assessed by an enzyme-linked immunosorbent assay (ELISA; Cooper, Trinkfield, Zanella, and Booth, 1989).

Cortisol shows both circadian and annual variation (Coe and Levine, 1885), especially in diurnal species (Janssens, Helmond, and Wiegant, 1995). Cortisol is secreted in a pulsing fashion, resulting in a possible circadian rhythm that must be considered when using cortisol as a measure of stress. In humans, the circadian rhythm of free cortisol in plasma and saliva correlate significantly (Kahn et al., 1988; Hiramatsu, 1981). Cortisol research with gorillas has found comparable results to those of humans, including a peak in the morning and a normal diurnal decline (Kuhar, Bettinger, and Laudenslager, 2005). Other animals found to have a similar diurnal decline are pigs (Klemcke, Nienaber, and Hahn, 1989), bulls (Thun, Eggenberger, Zerobin, Lüscher, and Vetter, 1981), giant pandas (Owen, Czekala, Swaisgood, Steinman, and Lindburg, 2005),

rhesus macaques (Quabbe, Gregor, Burme-Vogt, and Härdel, 1982), and Asian elephants (Bettinger and Laudenslager, 1998).

However, some species do not display cortisol circadian rhythms. Episodic but not circadian variations were found in cortisol levels in dogs (Koyama, Omata, and Saito, 2002; Kemppainen and Sartin, 1984), possibly because they are not diurnal animals and animals tend to exhibit their highest cortisol levels approximately when they wake up and begin locomotor activity (Cross and Rogers, 2001). Further tests may need to examine cortisol levels over wider time periods. When plasma cortisol was examined in sheep, there were mixed results that were not clarified until samples were taken every ten minutes for 24 hours (Fulkerson and Tang, 1979). This method found an ultradian rhythm with a peak just after midnight and nadir in the afternoon. In addition, stressors can change the cortisol circadian rhythm the day after the stressor occurs, depending on age and time of stressor (Ruis, Brake, Engel, Ekkel, Buist, Blokhuis, and Koolhaas, 1997). Furthermore, chronic stress can suppress the circadian rhythm (Möstl and Palme, 2002). For instance, pigs raised in barren conditions have blunted circadian rhythms in salivary cortisol when compared to pigs raised in enriched environments (de Jong, Prella, van de Burgwal, Lambooj, Korte, Blokhuis, and Koolhaas, 2000).

Performing research on stress using serum presents methodological issues because the sampling itself can induce stress and thus activate the HPA axis, especially if restraint is used (Kobelt, Hemsworth, Barnett, and Butler, 2003). This potential activation requires samples to be obtained quickly and eliminating the possibility of short intersample intervals (Boyce, Champoux, Suomi, and Gunnar, 1995). Short intersample intervals have been accomplished in some studies of farm animals using a jugular

catheter (e.g. Klemcke, Nienaber, and Hahn, 1989; Elvidge, Challis, Robinson, Roper, and Thorburn, 1976). However, this method is impractical for captive exotic animals. For zoo animals, positive reinforcement training for blood draw procedures is possible to at least reduce the cortisol response (Prescott, Buchanan-Smith, and Rennie, 2005), but is often complicated and time consuming, especially for multiple individuals (Kuhar, Bettinger, and Laudenslager, 2005).

Many studies have been performed using urinary or fecal cortisol and correlating those values with serum cortisol levels (Wasser, Hunt, Brown, Cooper, Crockett, Bechert, Millspaugh, Larson, and Monfort, 2000), although methods to measure cortisol in urine and feces is a somewhat recent development (see Goymann, Möstl, Van't Hof, Est, and Hofer, 1999). Urinary and fecal cortisol is a non-invasive methodology that can be useful for measuring reactions to more significant and long-term changes in animals' lives, such as seasonal or annual changes or pregnancy (Foley, Papageorge, and Wasser, 2001).

However, two drawbacks of fecal and urinary cortisol measures are that the change in cortisol may occur hours after the stressful event, limiting the real time significance of the measure, and a limited number of samples are available. The time lag may be problematic because the urinary and fecal cortisol values are a summation of events over a particular time period, potentially up to 72 hours (Kuhar, Bettinger, and Laudenslager, 2005). This time lag makes it impossible to examine acute stress using urinary or fecal cortisol. Harper and Austad (2000) found that increased fecal cortisol levels in three rodent species, house mice (*Mus musculus*), deer mice (*Peromyscus maniculatus*), and red-back voles (*Clethrionomys gapperi*), reflected stress experienced 6-12 hours before the sample was provided. Millspaugh, Washburn, Milanick, Beringer,

Hansen, and Meyer (2002) did not find a peak in fecal cortisol until 10-24 hours after ACTH trials in white-tailed deer (*Odocoileus virginianus*), versus the 30-60 minute reaction seen in salivary cortisol. Brown, Wemmer, and Lehnhardt (1995) examined serum and urinary cortisol levels in one African and one Asian elephant (*Loxodonta africana* and *Elephas maximus*) after an adrenocorticotrophin hormone (ACTH) challenge and found that serum cortisol increased four to eight times basal levels within 30 minutes after the first injection and begin to decline two to three hours after the third injection which came four hours after the first. Urinary cortisol increased in the first postinjection sample, which was limited to one sample provided 1.5-4 hours after the first injection, remained elevated 8 hours later and was back to baseline by the following morning. Stead, Meltzer and Palme (2000) examined serum and fecal cortisol levels after ACTH challenge in four juvenile African elephants. They found that serum cortisol levels increased four to seven times basal levels, with a highest value recorded two hours after injection. Fecal levels peaked approximately a full day after injections.

An additional challenge of using fecal cortisol levels is that lag time may be altered by season. For example, Morrow, Kolver, Verkerk, and Matthews (2002) found that mean lag time between plasma glucocorticoid concentration elevation and peak fecal concentrations after ACTH challenge in cattle decreased from 14.80 hours in the autumn when the animals were not lactating to 8.61 hours in the spring when they were lactating. This variability means that it is even harder to time sample collection for analysis.

### *Salivary Cortisol*

As early as 1959, it was recognized that saliva could be used to measure cortisol, but initially there were problems with sensitivity and appropriate assays (Vining,

McGinley, Maksvytis, and Ho, 1983). However, better methodology to measure cortisol in saliva was developed and using saliva cortisol levels has become a popular clinical method because of its numerous advantages over other methods (Vining and McGinley, 1984). Laudenslager, Bettinger, and Sackett (2006) report an exponential increase in the use of salivary cortisol in human research since 1989. Although it has only been validated in a few species, salivary cortisol is becoming an effective method with which to quantify cortisol levels (Millspaugh, et al., 2002), including the ability to track diurnal patterns and acute stress fluctuations (Kuhar, Bettinger, and Laudenslager, 2005). Saliva exhibits peak cortisol after a challenge only one to two minutes after peak plasma concentrations (Kirschbaum and Hellhammer, 1989). Lutz, Tiefenbacher, Jorgensen, Meyer, and Novak (2000) refer to urinary cortisol as a state estimate and salivary cortisol as a point estimate and highlight saliva's capacity for repeated sampling, even in short time intervals.

Using saliva to measure cortisol has many advantages, mainly its noninvasiveness and repeatability. It is also possible to measure cortisol from small amounts of saliva (de Weerth, Graat, Buitelaar, and Thijssen, 2003). In addition, although cortisol may take several minutes to become evident in saliva, it shows less time lag than urine or feces, allowing for an accurate stress reaction measurement (Bigert, Bluhm, and Theorell, 2005). Even stressful daily experiences in humans were related to increased cortisol secretion (von Eck, Berkhof, Nicholson, and Sulon, 1996).

Kirschbaum and Hellhammer (2000) state that cortisol filters from blood to saliva quickly, generally beginning to appear within a minute and peaking only two to three minutes behind blood level. Saliva is also easy to store (Bigert, Bluhm, and Theorell,

2005; Garden and Hansen, 2005) and can be measured by minor alterations to commercially available radioimmunoassay kits (Kahn, Rubinow, Davis, Kling, and Post, 1988; Ferguson, 1987). The main caution when using salivary cortisol is to avoid sample contamination by either blood through oral injury or food consumption, with protein and milk able to increase salivary cortisol and fluids able to dilute saliva (Laudenslager, Bettinger, and Sackett, 2006).

### *Cortisol and Enrichment*

Only a few studies have examined the effects of enrichment on cortisol levels. Schapiro, Bloomsmith, Kessel, and Shively (1993) found that inanimate environmental enrichment and social housing did not effect cortisol levels in juvenile rhesus macaques (*Macaca mulatta*), but outdoor housing led to lower cortisol levels than indoor housing. Blue fox (*Alopex lagopus*) cubs that were socially housed in an enlarged cage system were found to have lower serum cortisol responses to ACTH challenge than those housed traditionally (Ahola, Harri, Kasanen, Mononen, and Pyykönen, 2000). Additionally, male cubs housed socially had enlarged adrenals and female cubs housed socially exhibited greater weight gain. Overall, the data are ambiguous as to the effect of environmental enrichment on pituitary-adrenal changes, perhaps because levels were low even before enrichment (Carlstead and Shepherdson, 2000). In one study of structural enrichment with mice, the enrichment altered the social organization and led to increased cortisol levels in certain individuals (Haemisch, Voss, and Gartner, 1994). However, in other cases, such as the Carlstead, Brown, and Seidensticker (1993) study where leopard cats with elevated cortisol levels when in barren cages near larger cats, were provided enrichment, which in this case included hiding places, led to decreased cortisol levels.

Boinski, Swing, Gross, and Davis (1999) found a similar plasma and fecal cortisol decrease when they provided environmental enrichment to brown capuchins (*Cebus apella*). Therefore, environmental enrichment may not decrease cortisol below baseline levels, but it may reduce cortisol when it is elevated by certain stressors. Thus, in situations that increase stress levels, enrichment may help alleviate some portion of the cortisol response.

#### *Stereotypic Behavior and Cortisol*

Stereotypic behaviors have been linked to cortisol levels in several species. Research has found that the pituitary-adrenal system is stimulated when the animal is unable to perform behaviors they are motivated to perform during aversive situations (Weiss, 1968). It has been suggested that animals try to achieve an optimal level of arousal (Toates, 2000). Given that the two main explanations for the development of stereotypies involve either a compensatory function to deal with a lack of or excess of stimulation; stereotypies should either increase or decrease arousal levels (Dantzer, 1986). Therefore, it is possible to test between these alternatives by examining the effects of performing stereotypies on the pituitary-adrenal activity. If stereotypies decrease arousal, pituitary-adrenal activity should decrease, but if they are stimulating and enhance arousal, pituitary-adrenal activity should increase, resulting in increased cortisol levels. The use of stereotypic behavior to increase or decrease stimulation is common in humans with developmental disorders such as autism (Azrin, Kaplan, and Foxx, 1973; Guess and Carr, 1991). Dantzer and Mormede (1983) found that stereotypic chain pulling in pigs decreased plasma cortisol levels in food deprived, frustrated pigs on an extinction schedule. They also found that removing the chain, and thus preventing

stereotypic behavior, led to higher cortisol during the time in which the pig was in the chamber without the chain, but not after the pig was removed. They conclude that these results imply that stereotypic behavior can decrease excess arousal. The 1983 study was a follow up to their 1981 study which examined the effects of presence of a chain on cortisol levels and also found that pulling on a chain decreased cortisol levels across the session, but in control sessions with no chain, cortisol levels did not fall below pre-session values. Another study by Bildsøe, Heller, and Jeppesen (1991) found that immobilizations and food restrictions increased stereotypic levels in female ranch minks. In addition, females that exhibited high stereotypic rates had lower baseline cortisol rates but demonstrated higher cortisol responses to being immobilized. This cortisol pattern is evidence of the coping value of stereotypic behavior. Therefore, stereotypic behavior may reduce arousal in animals, but this possibility has not been fully investigated.

### *Cortisol and Welfare*

Prolonged elevated cortisol, or chronic stress, can be detrimental to welfare (Brown, Wielebnowski, and Cheeran, 2008). Chronic stress has been shown to decrease fitness, with complications such as suppression of the immune system (Möstl and Palme, 2002) and decreased reproductive functioning (Young, Walker, Lanthier, Waddell, Monfort, and Brown, 2004). However, chronic stress can also depress basal cortisol levels, creating a situation in which stressed individuals can only be differentiated from unstressed individuals by monitoring responses to acute stressors (Brown, Wielebnowski, and Cheeran, 2008). Therefore, it is essential to identify individuals suffering from excessive stress to evaluate their well-being, perhaps through health and behavioral means.

Increased cortisol has been correlated with short-term, possibly mild, stressful events, such as being moved to spatially and socially restricted housing (Moreira, Brown, Moraes, Swanson, and Monteiro-Filho, 2007; Beerda, Schilder, Bernadina, van Hooff, de Vries, and Mol, 1999b), restraint for an experimental procedure (Fuchs, Kirschbaum, Benisch, and Bieser, 1997), or various stimuli such as loud noises and shocks (Beerda, Schilder, van Hooff, de Vries, and Mol, 1998; Dess, Linwick, Patterson, Overmier, and Levine, 1983; Hanson, Larson, and Snowdon, 1976). Thus, effects are seen from events ranging from those generally thought of as mild stressors, such as restraint, or those that may have wider effects, such as spatially or socially restricted housing. However, it can be difficult to correlate cortisol with well-being (Bettinger and Laudenslager, 1998). Bettinger and Laudenslager (1998) claim that a main problem is defining stress. A related problem that comes from the unclear definition is that many assume that a lack of stress equates to well-being, when some levels of stress may be beneficial, allowing the animal to produce a rapid response to a novel or dangerous situation (Brown, Wielebnowski, and Cheeran, 2008). Examples of possible beneficial stress would be moving into a new exhibit, being introduced to a conspecific or novel enrichment, which may cause a stress response, but are also positive experiences (Bettinger, Larry, Goldstein, and Laudenslager, 1997). In addition, cortisol levels often return to baseline rather quickly, especially after mild stressors (Crockett, Bowers, Sackett, and Bowden, 1993, Natelson, Ottenweller, Cook, Pitman, McCarty, and Tapp, 1988). Lower cortisol levels may also indicate that an animal is able to cope with the stress based on its behavioral attributes, with cortisol being more significant in acute stress situations. For example, Redbo (1993) found that tethering heifers led to higher cortisol concentrations for the first week,

but cortisol levels decreased by the second week. In the first week, the heifers were not observed to lie down, but it is impossible to determine if this behavioral change was based on increased arousal or the potential difficulty lying down because of the chain and concrete floor. As the animals adjusted to the situation during the eight week study, the acute stress response decreased, the level of stereotypic behavior decreased, and the animals began lying down again. Therefore, the decrease in cortisol levels may have indicated that the animals were adapting to the situation, reiterating that cortisol cannot be examined without behavioral data.

An additional complication in using cortisol to indicate welfare is that cortisol may show species and even individual differences, with early rearing experience, gender, or dominance status affecting cortisol response. For example, when Clarke, Mason, and Moberg (1988) examined behavioral and cortisol responses to stressful events in three macaque species, they found that rhesus macaques (*Macaca mulatta*) were the most active behaviorally but had the lowest cortisol response, crab eaters (*M. fascicularis*) displayed the most behavioral disturbances and had the highest cortisol response, while the bonnet (*M. radiata*) was the most passive behaviorally and had intermediate cortisol levels. A study by Carlstead, Brown, Monfort, Killens, and Wildt (1992) subjected eight domestic cats (*Felis catus*) to handling, caging, canvas restraining bags, and venipuncture, half of the cats responded with increased urinary cortisol and half with decreased urinary cortisol. Therefore, Bettinger and Laudenslager (1998) recommend using cortisol data in addition to behavioral and other physiological data. It is also necessary to take several samples from each individual to establish basal and peak cortisol levels to allow determination of whether a cortisol value is normal for an animal.

They suggest six samples, assuming consistent sampling times and conditions, should be sufficient to establish an individual animal's norm.

Stress often triggers behavioral changes and cortisol levels have been associated with stereotypic behavior (Carlstead, 1998; Mason, 1991). Bettinger, Larry, Goldstein, and Laudenslager (1997) call for further studies that correlate cortisol levels with behavior to clarify how well behavior may indicate stress. A study of Asiatic elephants found that relocation led to an approximately 400% increase in stereotypic behavior and a corresponding cortisol increase of approximately 360% in fecal cortisol levels (Laws et al., 2007). Additionally, sleeping patterns were disturbed by the relocation. These results suggest that the relocation was stressful, and thus fecal cortisol levels were a good indicator of the decreased welfare accompanying this stressful event. Wilson, Bloomsmith, and Maple (2004) performed a study to examine the association between behavior and cortisol in three African elephants (*Loxodonta Africana*) using serum cortisol and behavioral indicators of stress. The authors mainly examined stereotypic swaying, studying whether it was more prevalent before regularly scheduled events, mainly shifting into the barn at night, and whether the elephants with higher rates of stereotypic swaying also had lower mean serum cortisol based on weekly blood draws. Observations were split between hour long observations during times of no interaction with the keepers and fifteen minute observations directly before the animals were released from or allowed into the barn (Shift observations). Stereotypic swaying was recorded in two of these elephants and accounted for a mean of 0.4% of the hour long observations and a mean of 18% of the shift observations, suggesting that regularly scheduled husbandry occurrences may elicit stereotypic behavior. An additional finding

was that more stereotypic swaying was seen in the afternoon prior to being shifted into the barn for the evening than in the morning prior to being shifted into the exhibit. There were individual differences in mean serum cortisol values, but those values did not correspond with the amount of stereotypic swaying. Results from this study inspired a deeper examination of the possible functions of stereotypic behavior, expanding to an investigation only possible through the use of salivary cortisol because of its real time utility. The current study also explored the effects of other behaviors and husbandry events on elephant cortisol.

In terms of links between stress and cortisol in elephants, Foley, Papageorge, and Wasser (2001) found increased cortisol levels in free-ranging African elephants during the dry season, relating to rain level and especially in larger groups. During the dry season, food and water were limited and body condition was seen to decline. These results suggest that cortisol values were an effective measure of the stress of competition for scarce resources. Additionally, cortisol levels were higher in lower ranking females, especially in larger groups, perhaps because they had more limited access to resources. This finding further highlights the link between stress and cortisol.

In a study comparing introduction methods for African elephants, Burks et al. found that the introduction method based on intuition of managers (“nonsequential”) led to higher cortisol values and greater submissive behaviors than the introduction method based on systematic data (“sequential”). These results suggest that submissive behaviors in elephants may relate to stress. For this study, submissive behaviors included avoidance, backing up, and screaming. However, one caveat from this study is that all of the sequential introductions came after the nonsequential, and thus the possibility exists

that the elephants had habituated to introductions. The authors address this point by stating that the introduction phases were separated by months and similar response patterns were seen. However, additional research is needed to examine the link between submissive behaviors and cortisol levels.

Bettinger, Larry, Goldstein, and Laudenslager (1997) found that salivary baseline cortisol levels in Asian elephants (*Elephas maximus*) were below the sensitivity levels for the radioimmunoassay procedure they used, meaning that salivary cortisol was only measurable when the subjects had elevated cortisol. Dathe, Kuckelkorn, and Minnemann (1992) were able to measure salivary cortisol in Asian elephants by modifying the RIA procedure by using less tracer and antiserum. They found a three fold increase in salivary cortisol when they introduced an unfamiliar Asian elephant into a herd. However, after a day the cortisol returned to baseline levels. They also observed a short-term increase in salivary cortisol that corresponded to a change in chaining procedures. A similar study by Schmid, Heistermann, Ganslöber, and Hodges (2001) measured behavioral and urinary cortisol changes following introduction of three female Asian elephants. They found that two of the elephants performed more stereotypic behavior, while one showed a decrease in stereotypic behavior. In addition, all three increased social behavior after the introduction. They also found increases in urinary cortisol of the two elephants they could reliably measure.

Cortisol levels in elephants have not been well explored. Similarly, salivary cortisol in elephants has been relatively unstudied as well. Fortunately, salivary assays are now more sensitive (0.003 µg/dl versus approximately 0.01 µg/dl) and salivary cortisol studies can be easily pursued with elephants (Laudenslager, personal

communication). Salivary cortisol has not been fully validated in elephants, and it has never been used to track real-time changes to mild stressors, mainly spatial restriction with a dominant conspecific. Salivary cortisol has been used to examine significant changes in elephant's lives, namely introductions. However, salivary cortisol has not been used to examine how cortisol changes in elephants in response to husbandry events and stereotypic behaviors. Systematic examination of these relationships assists in the continuing debate on elephant welfare.

### *Study Purpose*

The purpose of this study was to establish a baseline value for salivary cortisol in African elephants (*Loxodonta Africana*) and to use that baseline to examine the relationship between cortisol and welfare in African elephants. This relationship may help elucidate the function of the swaying behavior, whether it serves to decrease or increase arousal, as indicated through cortisol levels. This study also analyzed the effects of enrichment, training, and a mild stressor on cortisol. Behavioral data were also collected to determine rates of stereotypic behavior, current social relationships and dominance hierarchy, and how the elephants spend the majority of their time, including location in exhibit and proximity to conspecifics. These behavioral data were related to cortisol levels to look for parallel patterns that link cortisol and certain aspects of behavior, such as dominance, rates of stereotypic behavior, and whether the individual cycles or not.

### *Research Questions and Hypotheses*

1. How does salivary cortisol correlate to serum cortisol in African elephants?

HO: Salivary and serum cortisol relationships are not predictable.

- H1: Salivary cortisol values have a predictable relationship with serum values.
- 2 How do salivary cortisol values change over the day?
- HO: Salivary cortisol has no predictable circadian pattern.
- H1: Salivary cortisol has a predictable circadian pattern.
- 3 How does stereotypic swaying affect arousal levels, as measured by salivary cortisol?
- HO: Salivary cortisol is not affected by stereotypic swaying.
- H1: Salivary cortisol is decreased by stereotypic swaying.
- H2: Salivary cortisol is increased by stereotypic swaying.
- 4 How does the provision and use of enrichment affect arousal levels, as measured by salivary cortisol?
- HO: Salivary cortisol is not affected by enrichment.
- H1: Salivary cortisol is decreased by enrichment.
- H2: Salivary cortisol is increased by enrichment.
- 5 How do maintenance and novel training differ in their effects on salivary cortisol?
- HO: Maintenance and novel training have the same effect on salivary cortisol.
- H1: Maintenance and novel training have different effects on salivary cortisol
- 6 How does a mild stressor affect salivary cortisol levels?
- HO: Mild stressors do not affect salivary cortisol.
- H1: Mild stressors activate the HPA axis, thus increasing salivary cortisol.
- 7 How does behavior, mainly swaying rate, percent time spent consuming, activity level, and social behaviors (including aggression, dominance status, and affiliative behaviors), relate to cortisol levels?

HO: Behavior does not relate to cortisol levels.

H1: One or more of these measures of behavior correlates with cortisol levels.

- 8 How does behavior of these elephants now relate to the rates reported in Wilson, Bloomsmith, and Maple (2004)?

HO: Behavior has not changed since Wilson et al. (2004).

H1: One or more of the measures of behavior has changed since Wilson et al. (2004).

## METHOD

### *Subjects and Housing*

The subjects were three adult female African elephants (*Loxodonta Africana*), Kelly, Tara, and Dottie, who reside at Zoo Atlanta. These elephants were also the subjects of Wilson, Bloomsmith, and Maple (2004). All elephants were wild born. Kelly was born in approximately 1983, while Tara and Dottie were born in approximately 1982. All arrived at Zoo Atlanta and were introduced to each other in 1986. They were chained nightly until 1989. They are currently housed using a protected contact management system, differing from the free contact management system reported in Wilson, Bloomsmith, and Maple (2004) and Wilson, Bashaw, Fountain, Kieschnick, and Maple (2006). Each elephant is trained at least twice a day using portions of their diet besides hay and browse for reinforcement, which includes grain and produce, such as apples, lettuce, carrot, sweet potato, rutabaga, and melons.

The elephants in this study are housed in an exhibit that totals 2,414 m<sup>2</sup>. During the day, from approximately 0800 to 1730, these elephants are housed in naturalistic habitat that measures approximately 1,373 m<sup>2</sup> in size. This habitat consists of a pool,

shade structure, mud wallow, and logs for scratching and tusking. Additionally, the exhibit includes a barn, which is divided into an area accessible to visitors of 336 m<sup>2</sup> and another room of 164 m<sup>2</sup> which houses the elephant restraint device, and an outdoor paddock of 541 m<sup>2</sup> (Brockett, Stoinski, Black, Markowitz, and Maple, 1999). The elephants are brought indoors overnight, but often given access to the outdoor habitat or paddock if the temperature exceeds 40<sup>0</sup> F. Elephants are also frequently brought indoors during the day for training or separations necessary for husbandry reasons. Zoo visitors can view the elephants when they are in the naturalistic habitat or in the barn.

### *Materials and Methods*

General Behavioral Data: Continuous focal animal sampling was used to collect behavioral data. Observer software on a PDA was used to record behavior. Behavioral data were collected, during the same time periods as Wilson, Bloomsmith, and Maple (2004); 0815h-0945h (AM), 1130h-1300h (NOON), and 1600h-1730 (PM) to allow for comparisons of current rates of swaying behavior with those found in Wilson et al. Each elephant was observed as the focal animal for 30 minutes within those time periods for a total of fifteen hours per elephant, balanced across the time periods for each subject. Additional behavioral data were collected outside of those times periods for an additional fifteen am and fifteen pm 30 minute observations per elephant. For all observations, it was possible for the investigator to relocate to maintain visual access to the focal animal. The ethogram (see Table 1) used was adapted from Wilson, Bashaw, Fountain, Kieschnick, and Maple (2006). It included solitary, stereotypic, affiliative social, and agonistic social behaviors. In addition, proximity to conspecifics was also recorded to examine social relations (see Table 1).

Table 1. Elephant Behavioral Ethogram.

<u>Category</u>	<u>Behavior</u>	<u>Definition</u>
Solitary		
	Stand	Upright and stationary, not performing any other identified behavior
	Locomote	Non-stereotypic forward or backward movement over one body length, not performing any other identified behavior
	Rest	Lying down, alert or eyes closed
	Feed	Manipulation and consumption of food
	Drink	Ingestion of water
	Eliminate	Voiding feces or urine
	Self-directed	Spraying mud or dust on body, scratching body with foot or trunk or on any surface
	Obj Manipulation	Examination and manipulation of an object with trunk, mouth, or tusks
	Tusking	Digging with or rubbing of tusks on logs or other substrate
	Other	Any solitary behavior not listed on ethogram
Stereotypy		
	Sway	Standing, moving from side to side at least three times in a row
	Bite bars	Chewing or knowing on bars
	Head bob	Movement of the head up and down in a repetitive motion
	Pace	Moving around enclosure on a set path, at least three repetitions are required to consider a behavior pacing
	Trunk toss	Standing, extending and withdrawing trunk at least three times in a row
	Throw feces	Spraying fecal material on body or tossing it in the air
Affiliative or Neutral Social Behaviors		
	Approach	Movement to within one body length of conspecific
	Trunk touch	Contact of the trunk with a conspecific in a nonaggressive manner
	Twining trunks	Gentle overlapping of trunks with a conspecific
	Sentinel	Standing proximate or in contact with another elephant that is lying down for at least five seconds
	Leave	Movement to more than one body length away from conspecific without displacement
Agonistic Social Behaviors		
	Displace	Recipient moves one body length from current location within three seconds after conspecific approaches
	Avoid	Recipient moves one body length from current location prior to conspecific approach
	Drive	Proximate elephant places head against rump of conspecific and pushes
	Ear flap/threat	Ear held out perpendicular to head, oriented to conspecific, human, or change in the environment

Table 1. continued.

	Push	Lunging at a proximate conspecific from a stationary position and making contact with head or tusks on recipient
	Strike	Forceful contact of conspecific with body, trunk, object held by trunk, or foot
	Bump	Forceful pressure with the hip against conspecific
	Spar	Mutual head to head contact between two elephants using trunks, tusks, or head for at least five seconds
Proximity	Contact	Any part of elephant touching conspecific
	Proximity	Less than one body length from conspecific
	Distant	More than one body length from conspecific
Location	Area 1	Section of outdoor habitat farthest from barn, where training occurs
	Area 2	Section of outdoor habitat closest to water feature
	Area 3	Section of outdoor habitat closest to barn
	Area 4	Section of outdoor habitat through which elephants are shifted from barn to exhibit, elephants are not visible to the public
	Area 5	Large indoor area where elephants are viewed by public
	Area 6	Small indoor area where elephants are not visible to public
	Area 7	Outdoor paddock, not visible to public

Swaying Behavioral Data: Sway bouts were videotaped and, given that bouts appeared kinematically similar, a detailed analysis was performed on selected bouts. The analysis focused on weight shifting, foot placement, and head and trunk movement.

Baseline Cortisol: Tandem samples of blood and saliva were collected to establish a salivary cortisol baseline in the subjects (see Table 2). These subjects are trained to participate in regular blood withdrawals (Wilson, Bloomsmith, and Maple, 2004), however serum samples were limited to not subject the animals to excess venipuncture. In addition, they are trained to open their mouths on cue (personal observation), which allowed for saliva sampling. All samples were taken as quickly as possible, generally within one to two minutes, but always within five minutes from initiation of collection to prevent increased cortisol by activation of the HPA axis, especially for the serum samples.

Saliva samples were taken every other hour from 0730 to 1730 for three separate days (March 7<sup>th</sup>, 8<sup>th</sup>, and 9<sup>th</sup>, 2007) to gain a more complete understanding of the circadian rhythm during exhibit hours (Table 2, Baseline Saliva). Three of the samples on each day also included a tandem blood sample taken immediately after the saliva to prevent the saliva sample from being affected by a potential increase in cortisol due to blood withdrawal. However, one saliva sample each from Tara and Dottie were of insufficient volume to test. Two baseline test samples, (Table 2, Baseline Test Samples) were taken on separate days at the same times as the samples used to establish the circadian pattern. These samples, taken after the salivary and serum cortisol correlation was established, were used to test the predictions of the circadian pattern and also to test if saliva sampling predictability is stressful. In addition, three samples (Table 2, PM

samples) were distributed across 1600-1730, the times in which swaying was found to be more prevalent in Wilson, Bloomsmith, and Maple (2004). For these samples, the elephants were shifted into the barn early to obtain samples during the time in which the elephants tend to sway, but before they have begun the pre-shift sway. For all of the baseline samples, the elephants were sampled in the same order; Kelly, Dottie, Tara.

Table 2. Sample sizes of cortisol measurements by condition for each elephant.

	Kelly	Dottie	Tara	Total
Serum (Baseline only)	9	9	9	27
Baseline Saliva	18	17	17	52
Non-Baseline Saliva				
<i>Baseline Test Samples</i>	2	2	2	6
<i>Enrichment</i>	18	18	18	54
<i>Maintenance Training</i>	12	12	12	36
<i>Novel Training</i>	12	11	12	35
<i>Sway 1 minute</i>	5	0	5	10
<i>Sway 5 minute</i>	5	0	5	10
<i>Sway 10 minute</i>	5	0	5	10
<i>Sway 15 minute</i>	5	0	0	5
<i>Sway 30 minute</i>	5	0	0	5
<i>Mild Stressor</i>	4	4	4	12
<i>Social Housing</i>	10	0	10	20
<i>Ad lib</i>	2	1	3	5
<i>PM</i>	3	3	3	9
<i>Total non-baseline</i>	88	51	79	218
Total Saliva Samples	106	68	96	270

Behavior and Husbandry Determined Saliva: Baseline samples were taken and a significant correlation of salivary to serum cortisol was established before continuing the project. Therefore, although baseline samples were collected in March, the rest of the samples were collected between May 21, 2007 and July 10, 2008, with most samples collected between May and October of 2007. Additionally, all but the social housing samples were collected by mid-January 2008. Once a baseline was established, saliva samples were taken based on behavior and husbandry events (see Table 2). Only two elephants exhibit stereotypic swaying behavior; Kelly and Tara. Kelly engages in this behavior more often and for longer duration, thus more samples were collected from her. For Kelly, swaying bouts were interrupted, meaning that she was called over by care staff, and five samples each were taken at one, five, ten, fifteen and thirty minutes into the bout. Tara was placed in many situations to attempt to induce swaying, including putting her into the isolation stall and leaving her in the yard by herself. She generally would simply stand by the door or search for food. These sessions were repeated until five samples each were taken at one, five, and ten minutes into a swaying bout (see Table 2).

Samples were also taken from all three elephants after training and enrichment (see Table 2). These samples were taken fifteen and thirty minutes after training and enrichment to attempt to capture any effects. The training samples included twelve samples, six from 15 minutes after initiation of training and six from fifteen minutes after that, from six training sessions including only maintenance behaviors for each elephant. Additionally, twelve samples, six samples from 15 minutes after initiation of training and six from fifteen minutes after that, were taken from six training sessions that included

novel behaviors. However, one of Dottie's 30 minute samples was insufficient for testing, thus Dottie had only 11 novel training samples. These novel training samples allow for comparisons between standard and more challenging training sessions to determine if there is a difference in cortisol based on cognitive challenges (see, Dantzer and Mormède, 1983) All training sessions used positive reinforcement in a protected contact system and all of the sessions for this study took place in the elephant barn. Training sessions lasted fifteen minutes. The only elephant present was the individual being trained. The keeper doing the training for each session varied, but was recorded to determine possible affects on cortisol. Maintenance behaviors included simple behaviors, such as movements and placement of body parts, (e.g. placing a foot on a bar or raising their trunk), and more complicated behaviors (e.g. moving across the barn in waltz steps). All of these behaviors were reliably established. However, when the behavior had been trained varied. Each elephant was assigned one keeper to train its novel behavior. The novel behavior for Tara and Dottie was shaking the head from side to side in a manner that resembles a "no" response. Because Kelly already had been trained on this behavior, her novel behavior was to cross one rear leg over the other. These behaviors were trained using target poles that were faded out and replaced with a verbal cue by the sixth training session.

Eighteen enrichment samples were limited to collection after all elephants interacted with the items for at least 15 minutes (see Table 2). Nine samples were taken after the fifteen minutes of interaction. An additional nine samples were taken 30 minutes after the elephant began interacting with the enrichment item, although after the initial 15 minute interaction requirement the elephant could then walk away from the

enrichment area. Enrichment items were limited to food puzzles, including spin feeders and hay nets. Using only these items maintained consistency between enrichment items and these items are the ones the subjects interact with more (A. Stone, personal communication), thus allowing for the greatest possibility of decreasing cortisol. The food items offered did not include protein or milk, thus they are unlikely to have affected cortisol levels (Laudenslager, Bettinger, and Sackett, 2006).

Mild Stressor: To examine responses to mild stressors, each elephant was placed once in an isolation stall with its dominant conspecific for fifteen minutes. For these animals, dominance is circular, thus each elephant has another individual it is dominant to and one to which it is subordinate. Samples were collected at the beginning and end of the fifteen minute spatial restriction (see Table 2). In addition, samples were collected fifteen and thirty minutes after release, or 30 and 45 minutes after initial exposure. All of these sessions were observed for signs of aggression and would have been discontinued if any aggression occurred. For further examination of potentially stressful situations, samples were collected ad lib after an aggressive event between elephants or when elephants seemed especially stressed, based on behavioral indicators (see Table 2). This collection resulted in one sample from Kelly five minutes after she was struck by Dottie and two samples, one five and one ten minutes, after Tara was struck by Kelly. Additionally, one sample was collected from Dottie after chaining training for artificial insemination procedures and one sample was collected from Tara when she became agitated when she was alone in the yard and was running, vocalizing, and throwing sticks. Furthermore, samples were taken from Tara and Kelly first thing in the morning after Kelly was housed overnight with the other two subjects (five samples each) and

when Kelly was housed alone to give her a break and allow her full access to food overnight. These samples are referred to as the social housing samples.

Sample processing: The blood and cortisol samples were stored in a freezer until they were processed. They were then centrifuged at 2000 g for two minutes (Lamey and Nolan, 1994) and then kept frozen in O-ring sealed tubes at  $-20^{\circ}\text{C}$  or below until they were shipped to be assayed. Cortisol was measured by a commercial cortisol radioimmunoassay (RIA) kit by Dr. Mark Laudenslager, at the University of Colorado Health Sciences Center, Behavioral Immunology Laboratory, who has extensive experience in processing RIA kits, including their use for elephant cortisol, with an intra- and inter-assay coefficient of variation of less than 5% (Bettinger, Larry, Goldstein, and Laudenslager, 1997). A detailed description of the RIA procedure is available from Laudenslager, Bettinger, and Sackett (2006) and Laudenslager, Boccia Berger, Ruggles-Gennaro, McFerran, and Reite (1995). The minimum sensitivity of the test was 0.003, thus any cortisol lower than that value was coded as 0.003.

#### *Data Analysis*

Selected video taped swaying bouts for Kelly and Tara were examined to determine the swaying pattern of each elephant. The bouts were analyzed kinesthetically, meaning they were decomposed to determine the typical swaying pattern, including foot placement and type of head movement. This analysis resulted in a general description of the typical swaying pattern for each elephant.

General behavioral data were not normally distributed, thus nonparametric analyses were used. Effects of time and temperature on stereotypic and non-stereotypic activity were analyzed using Spearman correlations to ensure that time and temperature

were not confounding variables. Kruskal-Wallis and Mann-Whitney U tests were performed to determine any differences between elephants. Additionally, means and confidence intervals were used to determine how the elephants spent the majority of time and how stereotypic swaying has changed from Wilson, Bloomsmith, and Maple (2004).

Concentrations of cortisol in plasma and saliva were analyzed using a Spearman correlation coefficient because of a non-normal distribution. Salivary cortisol concentrations for all three elephants were plotted individually and as an average to determine the circadian trends and then analyzed through an autoregression analysis. Differences in cortisol during swaying were analyzed graphically and using sign tests. Cortisol levels during maintenance and novel training sessions were compared using sign test. Post-enrichment cortisol levels were also compared with the overall mean using a sign test. Each elephant acted as her own control to reduce the influence of individual variation (Palme, Robia, Baumgartner, Möstl, 2000), especially important in stress responsivity that can be very individualistic (Owen, Czekala, Swaisgood, Steinman, and Lindburg, 2005). An elephant population mean baseline cortisol value would not be useful because of the individual nature of cortisol, requiring a baseline to be established for each individual. Because each elephant served as her own control, power of the statistical tests is dependant on the number of samples taken and not on the number of elephants.

## RESULTS

General Behavioral Data: The behavioral data confirmed the anecdotal reports of circular dominance. Incidents of aggressive behaviors; mainly strike, drive, or displacement; tended to be performed in the anecdotally reported direction of dominance

(See Table 3). Behaviors, both aggressive and affiliative, were more frequent in the afternoon. Dottie struck Kelly three times, all in the afternoon. Kelly struck Tara once in the morning and once in the afternoon. Tara and Dottie were reported by animal care staff to have a closer relationship, which is supported by the absence of Tara and Dottie striking each other. However, Tara was observed to drive Dottie out of her way during two afternoon sessions. Dottie also drove Tara out of her way during one afternoon session. Dottie drove Kelly a total of ten times, three during morning sessions and seven during afternoon sessions. Displacements were observed more frequently. Dottie displaced Kelly 18 times in the morning and 35 in the afternoon, for a total of 53 times. Dottie also displaced Tara two times in pm sessions. Kelly never displaced Dottie, but she displaced Tara 8 times in am sessions and 16 in pm, for a total of 24 times. Tara displaced Kelly only once in the afternoon, but she displaced Dottie 11 times, also all in the afternoon.

Table 3. Incidents of aggressive behaviors recorded per elephant. All incidents are per the 60 half hour, 30 am and 30 pm, observations per elephant.

<b>Total</b>	am	pm	total
Sum of strike, kelly-tara	1	2	3
Sum of strike, dottie-kelly	0	3	3
Sum of drive, dottie-kelly	3	7	10
Sum of drive, dottie-tara	0	1	1
Sum of drive, tara-dottie	0	2	2
Sum of displaced, kelly-tara	8	16	24
Sum of displaced, dottie-kelly	18	35	53
Sum of displaced, dottie-tara	0	2	2
Sum of displaced, tara-kelly	0	1	1
Sum of displaced, tara-dottie	0	11	11

Affiliative behaviors were also observed at differing rates, which highlighted the closer relationship between Dottie and Tara. A major display of affiliative behavior was trunk touching (see Table 4). Dottie and Tara displayed this behavior 16 times, 9 in the am and 7 in the pm. Kelly and Tara displayed this behavior 4 times, 2 in the am and 2 in the pm. Kelly and Dottie were observed to touch trunks only one time in the pm.

Affiliative differences were also seen in the number of times each elephant approached and left the others (see Table 5). Each elephant approached its dominant less than it left its dominant and less than it approached the non-dominant elephant. Kelly approached Dottie 82 times, approached Tara 148 times, left Dottie 149 times, and left Tara 110 times. Tara approached Kelly 59 times, approached Dottie 141 times, left Kelly 91 times, and left Dottie 85 times. Dottie approached Tara 113 times, approached Kelly 141 times, left Tara 177 times, and left Kelly 74 times. Dottie and Tara spent a higher percentage of time proximate, 48.5% (std dev=24.03%) and 45.1% (std dev=23.50%) respectively, than Kelly, 32.9% (std dev=23.67%, see Table 6).

Table 4. Count of occurrences of trunk touching behavior behaviors recorded per elephant. All counts are per the 60 half hour, 30 am and 30 pm, observations per elephant.

	am	pm	Total
Sum of trunk, kelly-tara	2	1	3
Sum of trunk, dottie-kelly	0	1	1
Sum of trunk, dottie-tara	2	2	4
Sum of trunk, tara-kelly	0	1	1
Sum of trunk, tara-dottie	7	5	12

Table 5. Count of times one elephant approached or left another. All counts are per the 60 half hour, 30 am and 30 pm, observations per elephant.

	am	pm	Total
Sum of Approach, kelly-dottie	29	53	82
Sum of Approach, kelly-tara	49	99	148
Sum of Approach, dottie-kelly	48	93	141
Sum of Approach, dottie-tara	44	69	113
Sum of Approach, tara-kelly	20	39	59
Sum of Approach, tara-dottie	50	91	141
Sum of leave, kelly-dottie	55	94	149
Sum of leave, kelly-tara	46	64	110
Sum of leave, dottie-kelly	27	47	74
Sum of leave, dottie-tara	71	106	177
Sum of leave, tara-kelly	22	69	91
Sum of leave, tara-dottie	31	54	85

Table 6. Means and standard deviations of time spent in contact, proximate to, and distant from another elephant. All means are for the 60 half hour observations per elephant.

Elephant	Proximity Level	Mean	Standard Deviation
Kelly	Contact	0.13%	0.51%
	Proximate	32.94%	24.03%
	Distant	66.68%	24.10%
Dottie	Contact	0.65%	2.55%
	Proximate	48.48%	23.50%
	Distant	50.66%	23.31%
Tara	Contact	0.42%	1.04%
	Proximate	45.05%	23.67%
	Distant	53.07%	23.99%

Spearman correlations found no significant effects of time or temperature on non-stereotypic activity or stereotypic swaying (see Tables 7 and 8). Tests were also performed to examine differences in behavior by elephants (see Tables 9, 10 and Figure 1). None of the elephants differed in level of non-stereotypic activity ( $H_2=3.061$ ,  $p=.216$ ). Kelly spent 7.77% (std dev=6.17%) in non-stereotypic active behaviors, while Dottie spent 9.80% (std dev=7.79%) and Tara spent 7.29% (std dev=5.69%). Kruskal-Wallis tests revealed that percent duration of consume ( $H_2=12.873$ ,  $p=.002$ ), throwing feces ( $H_2=6.148$ ,  $p=.046$ ), and swaying ( $H_2=97.519$ ,  $p<.001$ ) did differ among elephants. Kelly spent significantly less time consuming than Dottie ( $U=1161.5$ ,  $p=.001$ ) and Tara ( $U=1290$ ,  $p=.007$ ) at 52.96% (std dev=32.16%). Tara spent the most time consuming at 73.42% (std dev=24.18%), which was not significantly different from Dottie who spent 69.59% (std dev=25.67%) of the time consuming ( $U=1627$ ,  $p=.364$ ). Tara ( $\bar{x}=1.70\%$ , std dev=4.93%) threw feces more than Kelly ( $\bar{x}=.0851\%$ , std dev= 2.61%;  $U=1537$ ,  $p=.011$ ), but not more than Dottie ( $\bar{x}=0.382\%$ , std dev=2.33%  $U=1648$ ,  $p=.081$ ). Kelly and Dottie also did not differ in time spent not throwing feces ( $U=1714.5$ ,  $p=.470$ ). Kelly swayed 24.77% (std dev= 26.16%) of the time, which was more than Tara who swayed only 0.0225% (std dev=0.174%;  $U=600$ ,  $p<.001$ ) of the time and Dottie who was never observed to sway ( $U= 610$ ,  $p<.001$ ). Percentage of time swaying during the times used by Wilson, Bloomsmith, and Maple (2004) was also calculated. Tara never swayed during these times and Kelly swayed 28.27% (std dev=26.91%).

Table 7. Spearman correlations values for the effects of temperature on non-stereotypic activity and stereotypic swaying for each elephant.

Elephant	Behavior	Spearman Correlation Coefficient of temperature	p-value
Kelly	Nonstereotypic Activity	-0.219	0.093
	Swaying	-0.131	0.319
Dottie	Nonstereotypic Activity	-0.061	0.645
Tara	Nonstereotypic Activity	-0.168	0.200
	Swaying	0.190	0.145

Table 8. Spearman correlations values for the effects of time on non-stereotypic activity and stereotypic swaying for each elephant.

Elephant	Behavior	Spearman Correlation Coefficient of time	p-value
Kelly	Nonstereotypic Activity	0.033	0.805
	Swaying	-0.131	0.319
Dottie	Nonstereotypic Activity	0.181	0.167
Tara	Nonstereotypic Activity	0.014	0.913
	Swaying	0.113	0.391

Table 9. Mean percent of activity budget spent on selected behaviors by elephant. All percentages are across the 60 half hour observations per elephant.

Elephant	Behavior	Mean	Standard Deviation
Kelly	Consume	52.96%	32.16%
	Non-stereotypic Activity	7.77%	6.17%
	Sway	24.77%	26.16%
	Throw Feces	0.09%	2.61%
Dottie	Consume	69.59%	25.67%
	Non-stereotypic Activity	9.80%	7.79%
	Sway	N/A	N/A
	Throw Feces	0.38%	2.33%
Tara	Consume	73.42%	24.18%
	Non-stereotypic Activity	7.29%	5.69%
	Sway	0.02%	0.17%
	Throw Feces	1.70%	4.93%

Table 10. A summary of behavior differences between elephants. All differences are based on comparisons between 60 thirty minutes observations per elephant.

Behavior	Comparison	Mann-Whitney U	p value
Sway			
	Kelly>Dottie	610	<.001
	Kelly>Tara	600	<.001
	Dottie=Tara	1770	0.317
Consume			
	Kelly<Dottie	1161.5	0.001
	Kelly<Tara	1290	0.007
	Dottie=Tara	1627	0.364
Throw Feces			
	Kelly=Dottie	1714.5	0.470
	Kelly<Tara	1537	0.001
	Dottie=Tara	1648	0.081
Proximity			
	Kelly<Dottie	1144.5	0.001
	Kelly<Tara	1231.5	0.003
	Dottie=Tara	1672	0.502

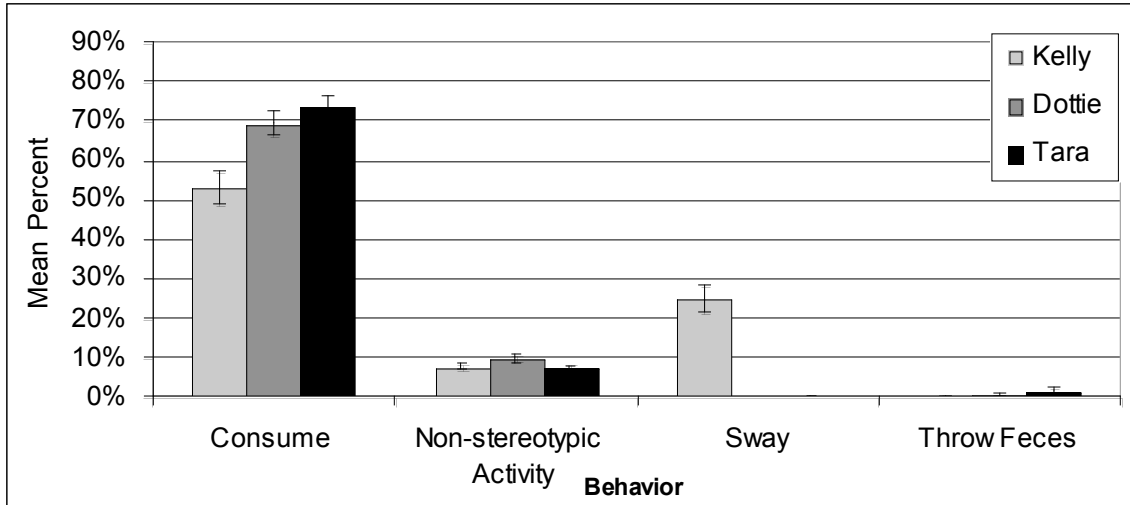


Figure 1. Mean percentage (+/- SEM) of selected behaviors by elephant. All percentages are based on 60 half hour observations per elephant. See Table 10 for significant differences.

Behavioral data also revealed differences in where the elephants spent the majority of their time (see Table 11). Kelly ( $\bar{x}=76.54\%$ , std dev=20.98%) was out in the exhibit visible to the public less than Dottie ( $\bar{x}=87.57\%$ , std dev=19.71%;  $U=1091.5$ ,  $p<.001$ ) or Tara ( $\bar{x}=91.10\%$ , std dev=16.15%;  $U=924.0$ ,  $p<.001$ ). Dottie and Tara did not differ ( $U=1658.5$ ,  $p=.458$ ). Kelly ( $\bar{x}=20.19\%$ , std dev=19.02%) spent significantly more time in the location in which she and Tara swayed, than Dottie ( $\bar{x}=10.67\%$ , std dev=18.64%;  $U=1130$ ,  $p<.001$ ) or Tara ( $\bar{x}=6.68\%$ , std dev=14.97%;  $U=864.5$ ,  $p<.001$ ). Dottie and Tara did not differ in the percentage of time they spent in this location ( $U=1499$ ,  $p=.071$ ). Time spent in this location, which is in front of the shift gate that separates the exhibit yard from the shift yard into the barn, often makes the elephant not visible to the public.

The elephants also varied in the amount of time they spent proximate to and distant from another elephant (see Table 6). Some of the time spent distant from another elephant was based on separations that occurred for husbandry reasons, but in general Kelly ( $\bar{x}=32.94\%$ , std dev=24.03%) spent less time proximate to another elephant, and therefore more time distant, than Dottie ( $\bar{x}=48.48\%$ , std dev=23.50%;  $U=1144.5$ ,  $p=.001$ ) or Tara ( $\bar{x}=45.40\%$ , std dev=23.67%;  $U=1231.5$ ,  $p=.003$ ) did. Dottie and Tara did not differ in amount of time spent proximate ( $U=1672$ ,  $p=.502$ ).

Table 11. Means and standard deviations of time spent in locations around the exhibit. All means are based on 60 half hour observations per elephant.

Elephant	Location	Mean	Standard Deviation
Kelly	On Exhibit Visible Areas (Area 1 and 2)	76.54%	20.98%
	On Exhibit Not Visible Area (Area 3, at gate where swayed)	20.19%	19.02%
Dottie	On Exhibit Visible Areas (Area 1 and 2)	87.57%	19.71%
	On Exhibit Not Visible Area (Area 3, at gate)	10.67%	18.64%
Tara	On Exhibit Visible Areas (Area 1 and 2)	91.10%	16.15%
	On Exhibit Not Visible Area (Area 3, at gate where swayed)	6.68%	14.97%

Swaying Behavioral Data: Tara and Kelly have qualitatively different swaying patterns. Kelly's swaying includes side-to-side head tilting with vertical movement of the head. Her trunk tends to hang down or occasionally she will hold it upright, but she does not actively swing it. Kelly's swaying does not involve a full weight shift, meaning that she keeps her weight mainly on the center of her body. Her chest does not re-center over her feet. Additionally, the majority of her each of the pads of her front feet remain on the ground. Kelly will also sway in varied locations, the gate that the elephants go through to shift into the barn is her most frequent location, but she will also sway farther back from the gate if other elephants are blocking it or even in other yard or barn locations. Tara, who spends less time swaying (see general behavioral data), and only at the shift gate, tends to perform a more active swaying motion. She is inclined to cross one leg over the other as she begins to sway in order to initiate a full weight shift of the front half of her body. The center of her mass tends to shift over her feet. She also includes side-to-side head rotation, more active than Kelly's head tilting. In addition, one of Tara's rear legs is often off of the ground, although she varies which one. Tara also exhibits more varied interbout behavior, with leg lifts, sniffing, and occasionally she may even walk in a circle and come back to swaying.

Correlation between saliva and serum: Both Dottie and Tara had one serum sample for which the saliva sample had to be discarded because of insufficient sample sizes. Therefore, a total of 25 serum and saliva pairs were collected, nine for Kelly and eight each for Tara and Dottie. Baseline serum ( $\bar{x}=1.824 \mu\text{g/dl}$ , std dev=1.127) and salivary cortisol ( $\bar{x}=0.04352 \mu\text{g/dl}$ , std dev=0.01996) values were significantly correlated ( $\rho=.792$ ,  $p<.001$ ,  $N=25$ ), therefore saliva is a valid measure of cortisol. Serum cortisol

values (see Table 8) were highest for Kelly ( $\bar{x}=2.734$   $\mu\text{g/dl}$ , std dev=1.238), intermediate for Dottie ( $\bar{x}=1.384$   $\mu\text{g/dl}$ , std dev= 0.758), and lowest for Tara ( $\bar{x}=1.241$   $\mu\text{g/dl}$ , std dev=0.598). Additional validation tests were run to ensure precision of the salivary cortisol tests. The average intra-assay coefficient of variation (CV) was 1.67%, consistent with Laugenslager's reported CV values below 5% (see Bettinger, Larry, Goldstein, and Laudenslager, 1997). Serial validation was also run to test specificity of the assay for elephant salivary cortisol. It was found that the serial dilution curve was parallel to the standard curve and the homogeneity of regression value was not significant, demonstrating that the slopes of the two regression lines are not different ( $F=0.501$ ,  $p=.485$ ,  $N=16$ ; see Figure 2). Therefore, this methodology demonstrates high specificity.

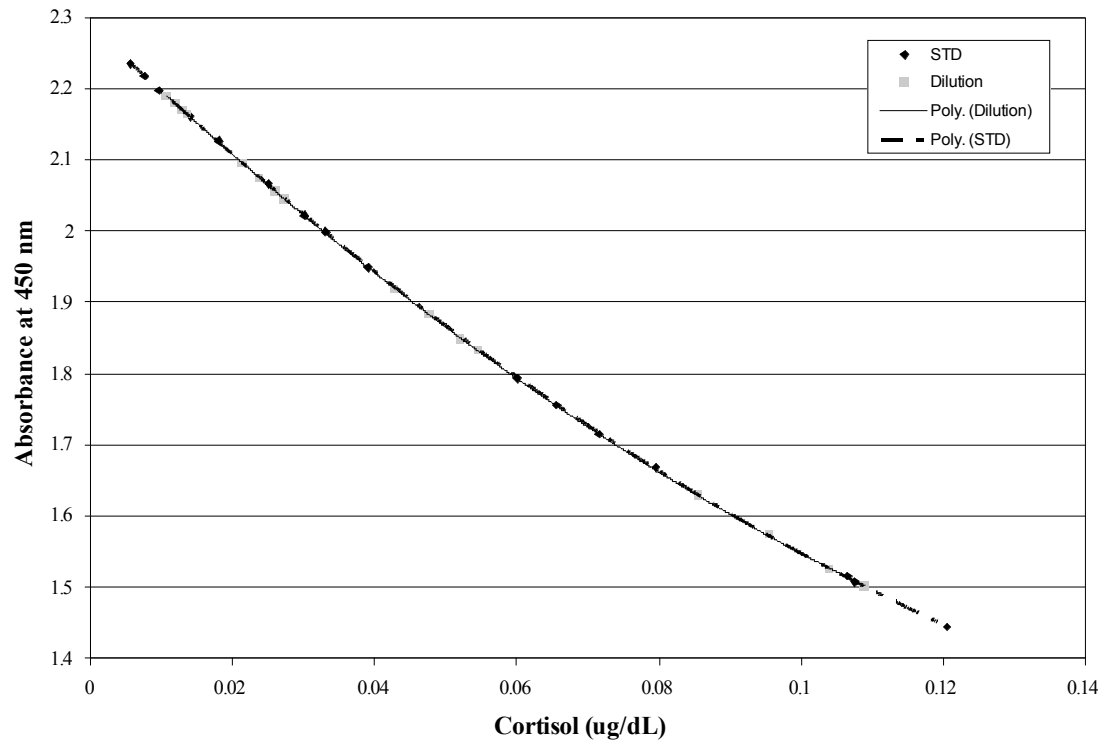


Figure 2. Parallelism of the standard and serial dilution (N=16) curves for salivary cortisol.

Baseline cortisol: Baseline salivary cortisol levels (see Table 12 and Figure 3) were highest for Kelly ( $\bar{x}=0.0540$   $\mu\text{g/dl}$ , std dev=0.0249). Intermediate levels were found for Dottie ( $\bar{x}=0.0340$   $\mu\text{g/dl}$ , std dev=0.0169). Tara had the lowest values at ( $\bar{x}=0.0300$   $\mu\text{g/dl}$ , std dev=0.00972). The means for Dottie and Tara fall outside of the 95% confidence interval for Kelly's mean (CI<sub>95</sub>= 0.041; 0.066). However, Tara and Dottie did not differ from each other based on confidence intervals (Dottie CI<sub>95</sub>=0.0253; 0.0427, Tara CI<sub>95</sub>=0.0250; 0.0350). The same results were obtained through Mann-Whitney U tests. Kelly's baseline salivary cortisol is significantly higher than Dottie's (U=78.0, p=.013, see Table 13) and Tara's (U=65.5, p=.004), but Dottie and Tara did not differ from each other (U=139.5, p=.863).

Baseline cortisol tended to decrease across the day for Tara and Dottie (see Figures 4-6, Table 14), but regression analyses did not yield any predictive equations, with all linear and logarithmic R<sup>2</sup> values below 0.4. Quadratic values were not much better; the highest was .468 for Tara, which is still not high enough for accurate prediction. Although there is no regression that allows prediction from time with confidence, there is a significant trend of decrease across the day for Dottie (R<sup>2</sup>=.40, p=.006) and Tara (R<sup>2</sup>=.281, p=.029), but not for Kelly (R<sup>2</sup>=.036, p=.449). However, an autocorrelational analysis revealed that Dottie's baseline cortisol values were autocorrelated. Her Durbin-Watson (d) statistic was 0.643, which is below the d<sub>l</sub> for 18 samples, which is 1.08. Therefore, her data were transformed to correct for autocorrelation, leading to a corrected R-square value of 0.276 (p=.037). The Durbin-Watson value for her transformed data was 1.712. For both Kelly and Tara baseline data were not autocorrelated (d<sub>Kelly</sub>=1.974; d<sub>Tara</sub>=1.965). Overall, Dottie and Tara had a

significant correlation between saliva and time, but Kelly did not. A visual inspection of the graphs of trends across the day for each elephant reveals that Kelly had more variable pm cortisol values (see Figures 4-6).

The baseline test samples, the two samples taken on separate days at the same times as the baseline samples, were lower than the baseline samples, with all of the baseline tests falling outside of the confidence intervals for baseline cortisol means. The baseline test values for Kelly were 0.027 and 0.015  $\mu\text{g}/\text{dl}$  (baseline mean 0.054  $\mu\text{g}/\text{dl}$ ), for Dottie they were 0.014 and 0.007  $\mu\text{g}/\text{dl}$  (baseline mean 0.034  $\mu\text{g}/\text{dl}$ ), and for Tara both test samples were 0.003  $\mu\text{g}/\text{dl}$  (baseline mean 0.03  $\mu\text{g}/\text{dl}$ ). However, these were also lower than the low end of the confidence intervals for the overall means. However, it is impossible to rule out the possibility that sample collection was stressful before it became routine or perhaps the predictability of the sampling was stressful.

Table 12. Means and standard deviations of cortisol ( $\mu\text{g}/\text{dl}$ ) by condition for each elephant. Total Saliva includes baseline and non-baseline saliva.

Elephant	Condition	Mean Cortisol ( $\mu\text{g}/\text{dl}$ )	Standard Deviation	n
<b>Kelly</b>				
	Baseline Serum	2.734	1.238	9
	Total Saliva	0.0557	0.0527	106
	Baseline Saliva	0.054	0.0249	18
	Non-baseline Saliva	0.0561	0.0568	88
	<i>Maintenance Training</i>			
		0.023	0.03	12
	<i>Novel Training</i>	0.065	0.066	12
	<i>Enrichment</i>			
		0.095	0.0811	18
	<i>Sway 1 min</i>	0.0468	0.0303	5
	<i>Sway 5 min</i>	0.0438	0.0219	5
	<i>Sway 10 min</i>	0.0196	0.02	5
	<i>Sway 15 min</i>	0.031	0.0252	5
	<i>Sway 30 min</i>	0.017	0.0126	5
<b>Dottie</b>				
	Baseline Serum	1.384	0.758	9
	Total Saliva	0.0313	0.0167	68
	Baseline Saliva	0.034	0.0169	17
	Non-baseline Saliva	0.0303	0.0167	51
	<i>Maintenance Training</i>			
		0.0193	0.0121	12
	<i>Novel Training</i>	0.038	0.0114	11
	<i>Enrichment</i>			
		0.0372	0.0171	18

Table 12 continued.

Elephant	Condition	Mean Cortisol ( $\mu\text{g}/\text{dl}$ )	Standard Deviation	n
Tara				
	Baseline Serum	1.241	0.598	9
	Total Saliva	0.0239	0.0162	96
	Baseline Saliva	0.03	0.00972	17
	Non-baseline Saliva	0.0226	0.017	79
	<i>Maintenance Training</i>	0.0075	0.00619	12
	<i>Novel Training</i>	0.028	0.086	12
	<i>Enrichment</i>	0.0301	0.0211	18
	<i>Sway 1 min</i>	0.0204	0.00702	5
	<i>Sway 5 min</i>	0.0152	0.00521	5
	<i>Sway 10 min</i>	0.0166	0.00945	5

Table 13. A summary of salivary cortisol differences between elephants by condition.

Baseline Salivary Cortisol		Mann-Whitney U	p value	n1/n2
	Kelly>Dottie	78	0.014	18/17
	Kelly>Tara	65.5	0.004	18/17
	Dottie=Tara	129.5	0.863	17/17
Non-Baseline Salivary Cortisol				
	Kelly<Dottie	1778.5	0.042	88/51
	Kelly<Tara	2112.5	<.001	88/79
	Dottie>Tara	1448.5	0.007	51/79
Total Salivary Cortisol				
	Kelly=Dottie	2700	0.005	106/68
	Kelly<Tara	2992.5	<.001	106/96
	Dottie>Tara	2433.5	0.006	68/96

Table 14. Summary of Spearman correlations of salivary cortisol and time by condition.

Baseline Salivary Cortisol	Elephant	Spearman Correlation	p value	n
	Kelly	0.036	0.449	18
	Dottie	0.400	0.006	17
	autocorrelation correction	0.276	0.037	17
	Tara	0.281	0.029	17
Non-Baseline Salivary Cortisol				
	Kelly	0.095	0.003	88
	Dottie	0.113	0.016	51
	Tara	0.139	0.001	79
Total Salivary Cortisol				
	Kelly	0.079	0.003	106
	Dottie	0.156	0.001	68
	Tara	0.122	<.001	96

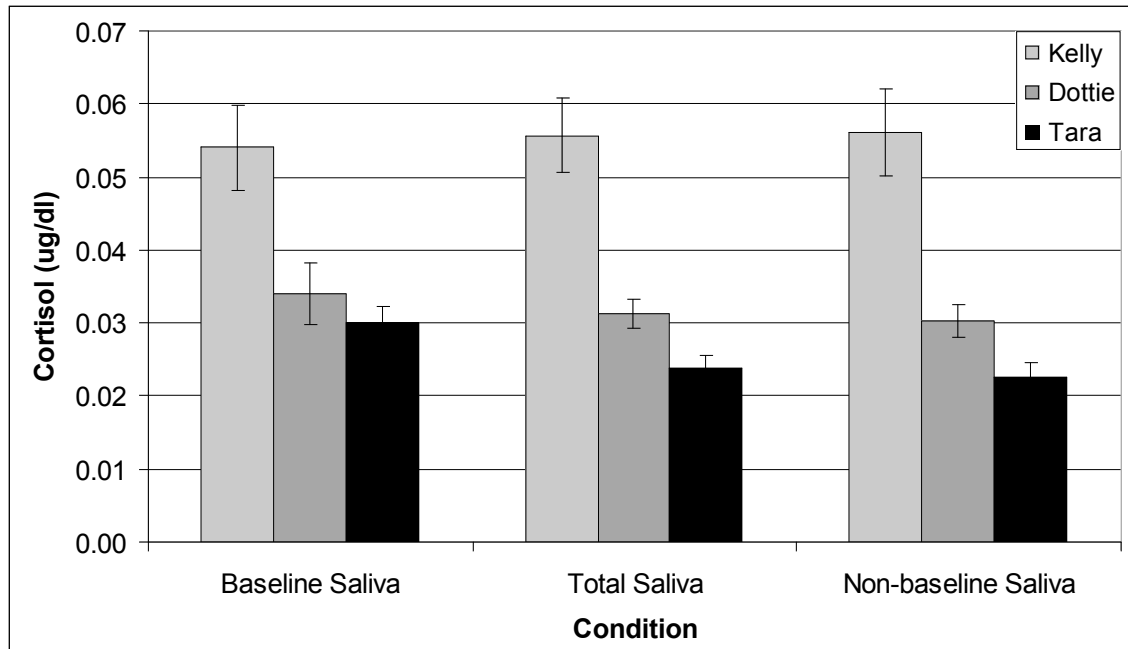


Figure 3. Mean (+/- SEM) salivary cortisol for each elephant by condition. Total saliva is baseline plus non-baseline saliva samples. For sample sizes per elephant see Table 2. For significant differences, see Table 13.

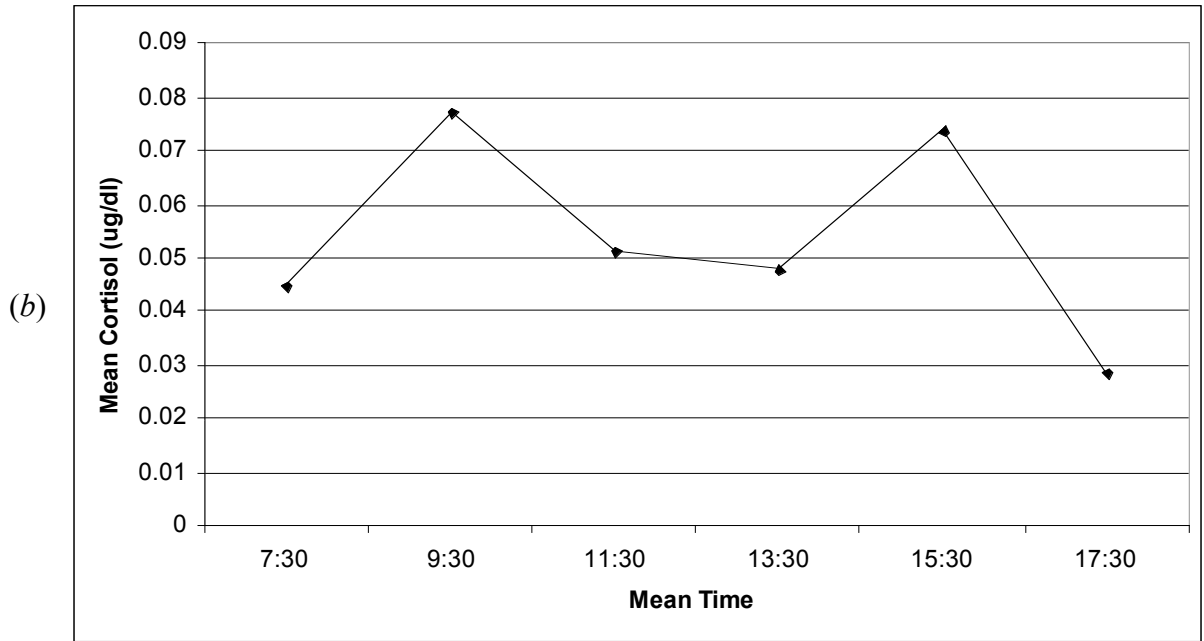
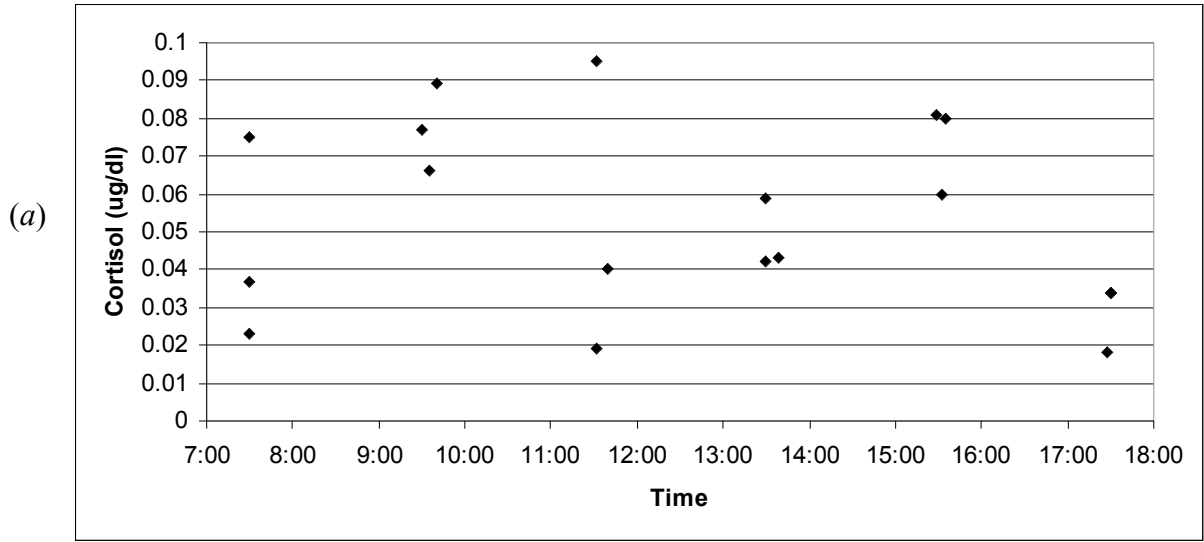


Figure 4. Individual data points (a) and mean (b) baseline salivary cortisol ( $\mu\text{g/dl}$ ) values across the day for Kelly.

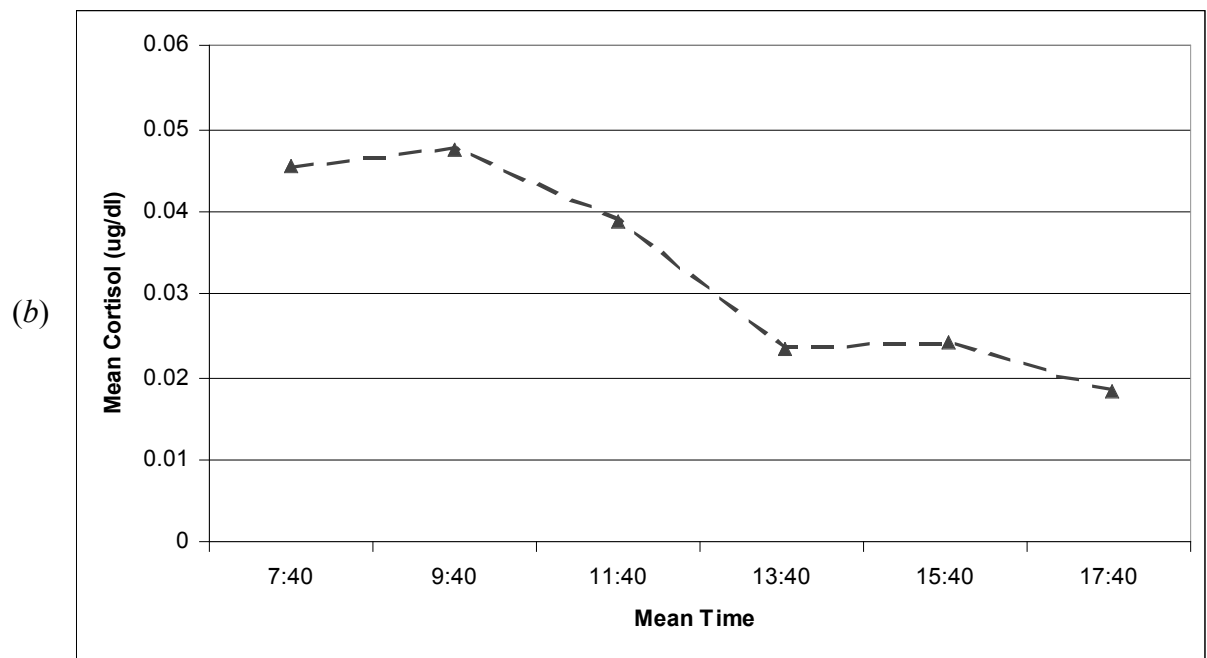
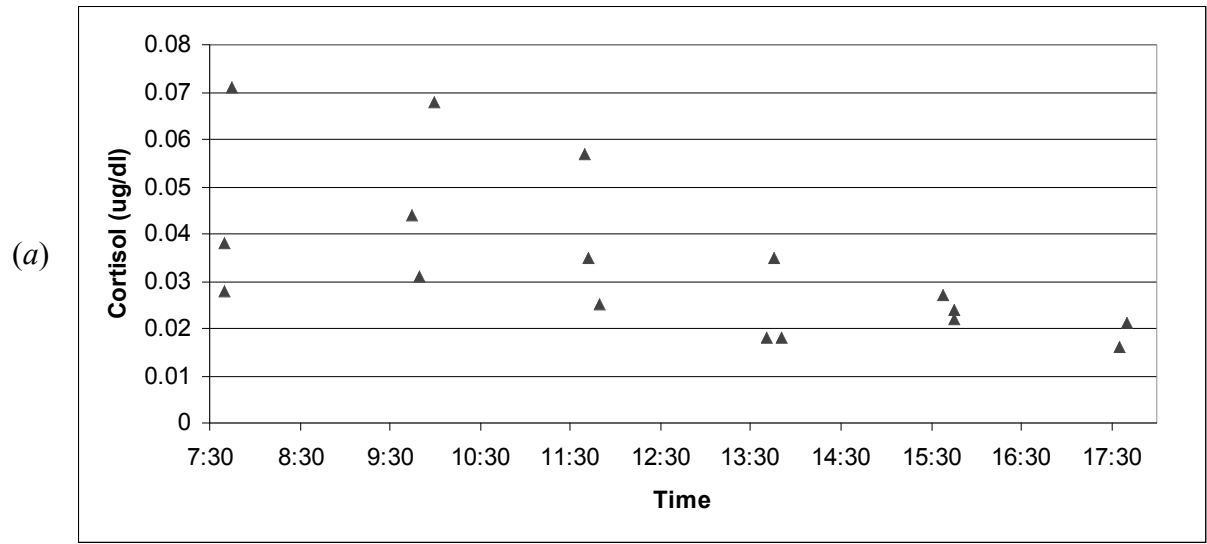


Figure 5. Individual data points (a) and mean (b) baseline salivary cortisol ( $\mu\text{g/dl}$ ) values across the day for Dottie.

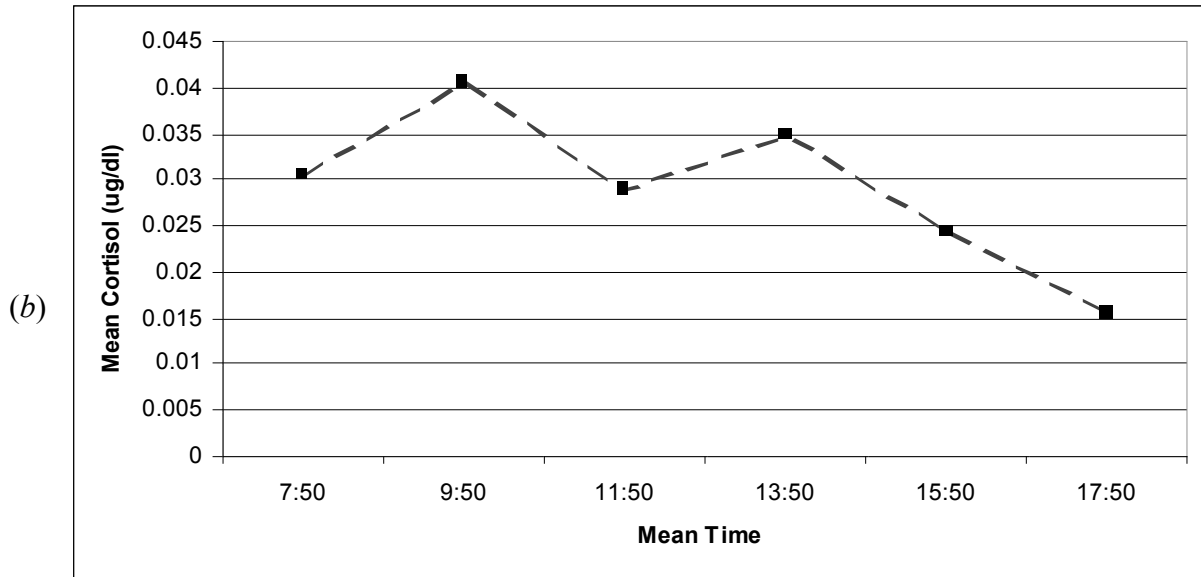
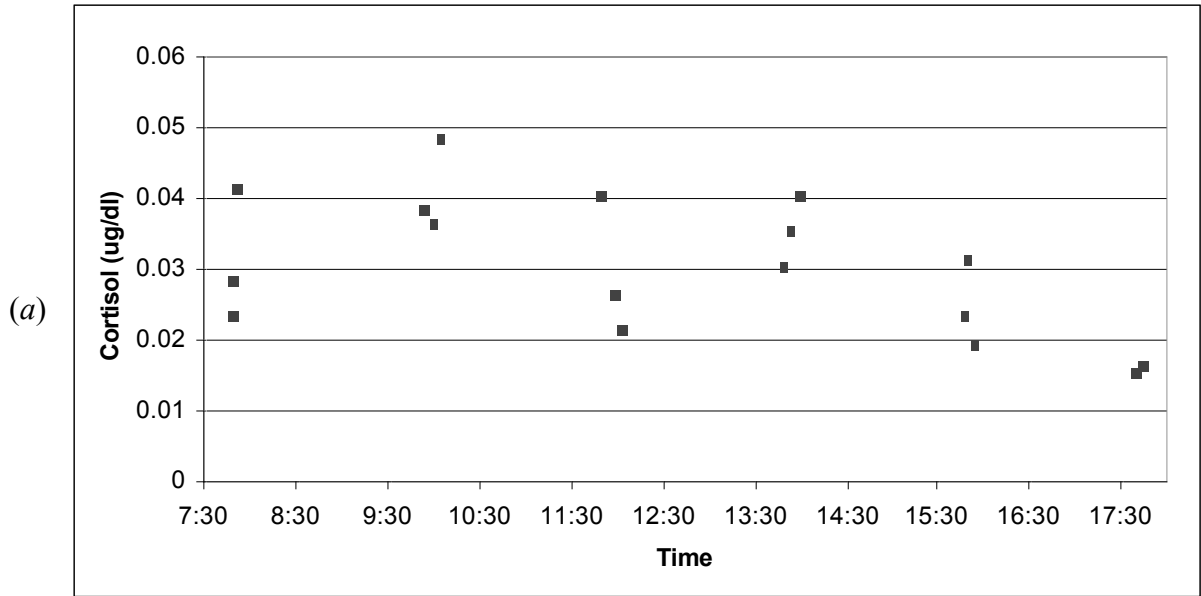


Figure 6. Individual data points (a) and mean (b) baseline salivary cortisol ( $\mu\text{g}/\text{dl}$ ) values across the day for Tara.

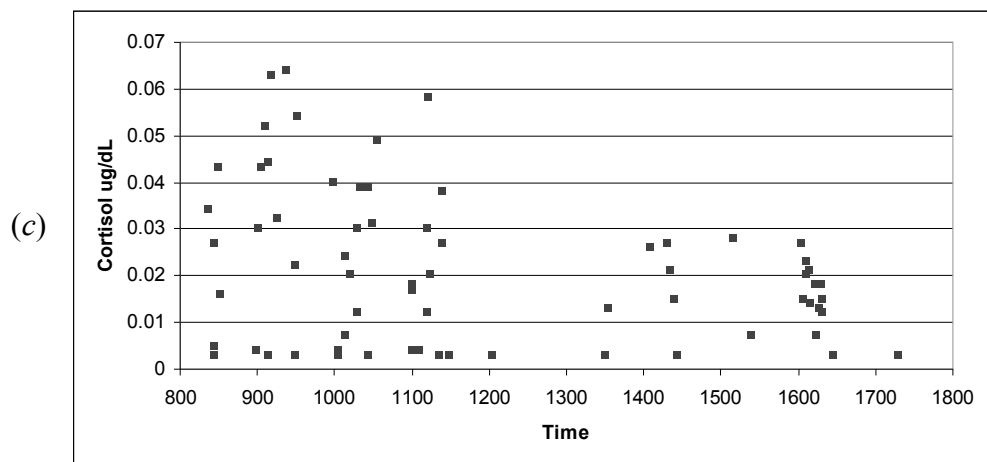
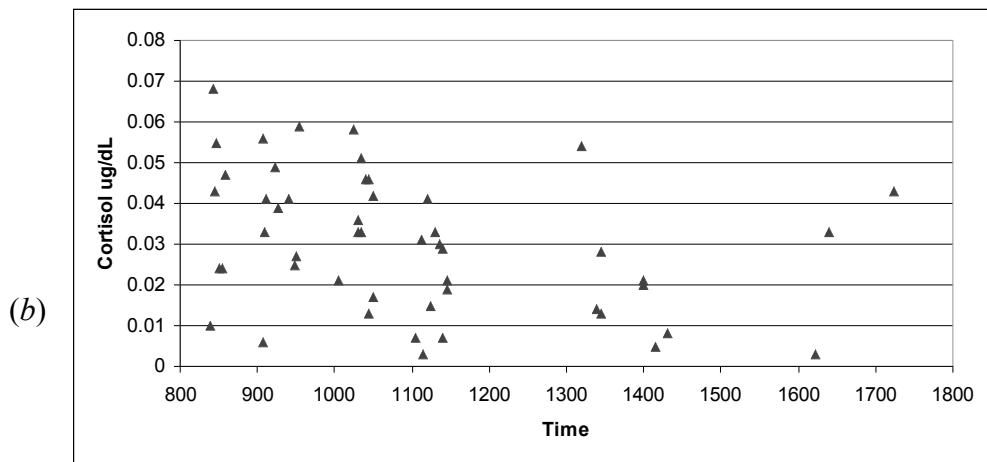
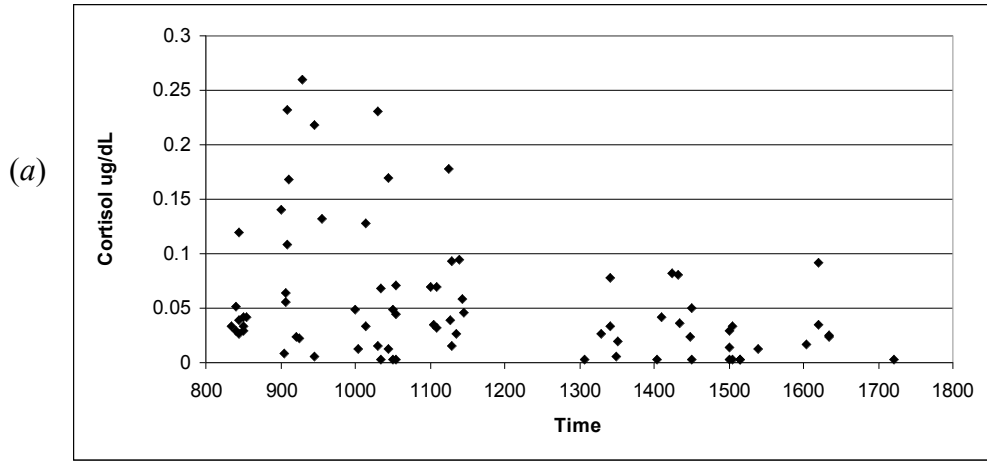


Figure 7. Non-baseline individual points for Kelly (a), Dottie (b), and Tara (c).

Behavior and Husbandry Determined Saliva: Total mean salivary cortisol, including baseline and non-baseline (see Table 12 and Figure 3), was highest for Kelly ( $\bar{x}=0.0557$   $\mu\text{g}/\text{dl}$ , std dev=0.0527,  $\text{CI}_{95}=0.0456$ ; 0.0659). Next highest was Dottie ( $\bar{x}=0.0313$   $\mu\text{g}/\text{dl}$ , std dev=0.0167,  $\text{CI}_{95}=0.0272$ ; 0.0353), with Tara exhibiting the lowest cortisol ( $\bar{x}=0.0239$   $\mu\text{g}/\text{dl}$ , std dev=0.0162,  $\text{CI}_{95}=0.0207$ ; 0.0272). Kelly's total cortisol was significantly higher than Dottie's ( $U=2700$ ,  $p=.005$ , see Table 13) and Tara's ( $U=2992.5$ ,  $p<.001$ ). Dottie's cortisol was also significantly higher than Tara's ( $U=2433.5$ ,  $p=.006$ ).

The baseline values did not differ significantly from the non-baseline for any of the three elephants (Kelly:  $U=646.5$ ,  $p=.221$ , Dottie:  $U=397.5$ ,  $p=.610$ , Tara:  $U=493.5$ ,  $p=.087$ ). There was a significant difference among the elephants in non-baseline salivary cortisol ( $H_2=21.457$ ,  $p<.001$ , see Tables 12 and 13). Kelly had the highest salivary cortisol ( $\bar{x}=0.0561$   $\mu\text{g}/\text{dl}$ , std dev=0.0568, range = 0.003 to 0.260). Dottie had the next highest cortisol values ( $\bar{x}=0.0304$   $\mu\text{g}/\text{dl}$ , std dev= 0.0167, range = 0.003 to 0.068). Tara had the lowest cortisol values ( $\bar{x}=0.0226$   $\mu\text{g}/\text{dl}$ , std dev= 0.0170, range = 0.003 to 0.064). Kelly had significantly higher cortisol than Dottie ( $U= 1778.5$ ,  $p=.042$ ) and Tara ( $U= 2112.5$ ,  $p<.001$ ). Additionally, Dottie's cortisol was significantly higher than Tara's ( $U=1448.5$ ,  $p=.007$ ).

Non-baseline saliva was lower in the pm than am for Kelly whose baseline data did not have a significant trend ( $U=427.5$ ,  $p<.001$ ) and Tara ( $U=418.5$ ,  $p=.01$ ), but not for Dottie ( $U=137$ ,  $p=.057$ ). However, Dottie only had 11 pm samples, versus 28 for Kelly and 24 for Tara. Perhaps additional samples for Dottie would have revealed a trend not evident in this amount of samples (See Figure 7). Non-baseline saliva decreased

throughout the day for all elephants (Kelly:  $R^2=0.095$ ,  $p=.003$ , Dottie:  $R^2=0.113$ ,  $p=.016$ , Tara:  $R^2=0.139$ ,  $p=.001$ , see Table 14). Total saliva, baseline plus non-baseline, also decreased throughout the day for all three elephants (Kelly:  $R^2=0.079$ ,  $p=.003$ , Dottie:  $R^2=0.156$ ,  $p=.001$ , Tara:  $R^2=0.122$ ,  $p<.001$ , see Table 14).

In terms of training, cortisol values for all three elephants were higher under novel training situations than maintenance training (see Table 12). For Kelly, the mean for maintenance training was  $0.023 \mu\text{g/dl}$  (std dev= $0.030$ ) and the mean for novel training was  $0.065 \mu\text{g/dl}$  (std dev= $0.066$ ). For Dottie, the mean for maintenance training was  $0.0193 \mu\text{g/dl}$  (std dev= $0.0121$ ) and the mean for novel training was  $0.038 \mu\text{g/dl}$  (std dev= $0.0114$ ). For Tara, the mean for maintenance training was  $0.0075 \mu\text{g/dl}$  (std dev= $0.00619$ ) and the mean for novel training was  $0.028 \mu\text{g/dl}$  (std dev= $0.0860$ ). There was no consistent pattern for the change in salivary cortisol between the sample taken after fifteen minutes of training and that taken an additional fifteen minutes later. Additionally, the salivary cortisol values for novel training did not differ from the means for each animal. However, the values were higher for maintenance training based on sign tests for all three animals (Kelly:  $p=0.039$ , Dottie:  $p=0.039$ , Tara:  $p<.001$ ). Additionally, novel training means were significantly higher than maintenance training based on sign tests for all three animals (Kelly:  $p=0.006$ , Dottie:  $p=0.001$ , Tara:  $p<.001$ ).

For Kelly, mean salivary cortisol during enrichment trials was  $0.095 \mu\text{g/dl}$  (std dev= $0.0811$ , see Table 12). Dottie's mean value was  $0.0372 \mu\text{g/dl}$  (std dev= $0.0171$ ). Tara's mean enrichment salivary cortisol was  $0.0301 \mu\text{g/dl}$  (std dev= $0.0211$ ). There was no consistent pattern for the change in salivary cortisol between the sample taken after fifteen minutes of interaction with the enrichment items and the sample taken an

additional fifteen minutes later. Additionally, these values do not differ significantly from the means for each individual elephant based on sign tests. However, many of Kelly's highest cortisol values were during enrichment trials. Three of Kelly's four highest values, the only four that were higher than 0.2 µg/dl, were during enrichment trials. Additionally, eight of the fourteen samples above 0.1 µg/dl were also enrichment samples. Unfortunately, given the method of sample collection and its interruption of behavior, behavioral observations were not recorded during salivary cortisol trials. Therefore, it is impossible to determine what differed between these high cortisol enrichment trials and those that were much lower.

In general, swaying reduces cortisol values (see Table 12 and Figure 8), but this effect is larger for Kelly than Tara. For Kelly, mean cortisol values were higher for one minute into a swaying bout ( $\bar{x}$ =0.0468 µg/dl, std dev=0.0303, range=0.019 to 0.081), than five minutes ( $\bar{x}$ =0.0438 µg/dl, std dev=0.0219, range=0.027 to 0.082), ten minutes ( $\bar{x}$ =0.0196 µg/dl, std dev=0.0200, range=0.003 to 0.029), fifteen minutes ( $\bar{x}$ =0.031 µg/dl, std dev=0.0252, range=0.003 to 0.068), or thirty minutes into a bout ( $\bar{x}$ =0.017 µg/dl, std dev=0.0126, range=0.003 to 0.023). Overall, swaying cortisol values ( $\bar{x}$ =0.031 µg/dl, std dev=0.0241) were significantly lower than Kelly's overall mean salivary cortisol as assessed using a sign test (p=.001). For Tara, the pattern was similar with the average at one minute into the bout ( $\bar{x}$ =0.0204 µg/dl, std dev= 0.00702, range=0.013 to 0.028) higher than those taken after longer amounts of swaying. The mean for five minutes into a bout was 0.0152 µg/dl (std dev=0.00521, range=0.007 to 0.021) and that for ten minutes was 0.0166 µg/dl (std dev=0.00945, range=0.003 to 0.027). Tara's overall swaying values ( $\bar{x}$ =0.0174 µg/dl, std dev= 0.00725) were also

lower than her overall salivary cortisol mean when examined using a sign test ( $p=.035$ ). Given that pm cortisol levels were lower than am and the majority, but not all, of sway samples were taken in the pm, sign tests were rerun to compare swaying cortisol to mean of pm values not including swaying values. The difference was still significant for Kelly ( $p=.043$ ), but not for Tara ( $p=.607$ ).

Samples could only be taken together a few times, as generally once an elephant's sway bout was interrupted, it did not resume swaying. For Kelly, this situation only happened one time in which a sample was taken one minute into a bout ( $0.081 \mu\text{g}/\text{dl}$ ) and then again ten minutes after she began to sway again ( $0.05 \mu\text{g}/\text{dl}$ ) which was eight minutes after her release from collecting the initial sample. For Tara, this situation happened twice. One occurrence was when the initial sample was taken after one minute ( $0.028 \mu\text{g}/\text{dl}$ ), and that sample was almost equivalent to the sample taken after ten minutes of swaying ( $0.027 \mu\text{g}/\text{dl}$ ) which began forty minutes after the initial sample was taken. On another occasion, Tara's cortisol value was slightly higher ( $0.020 \mu\text{g}/\text{dl}$ ) after swaying one minute than when she began swaying ten minutes after release and her cortisol was taken ten minutes into her bout ( $0.018 \mu\text{g}/\text{dl}$ ). Therefore, cortisol values were never found to rise while an elephant was engaged in a swaying bout, even if it was interrupted to collect a sample.

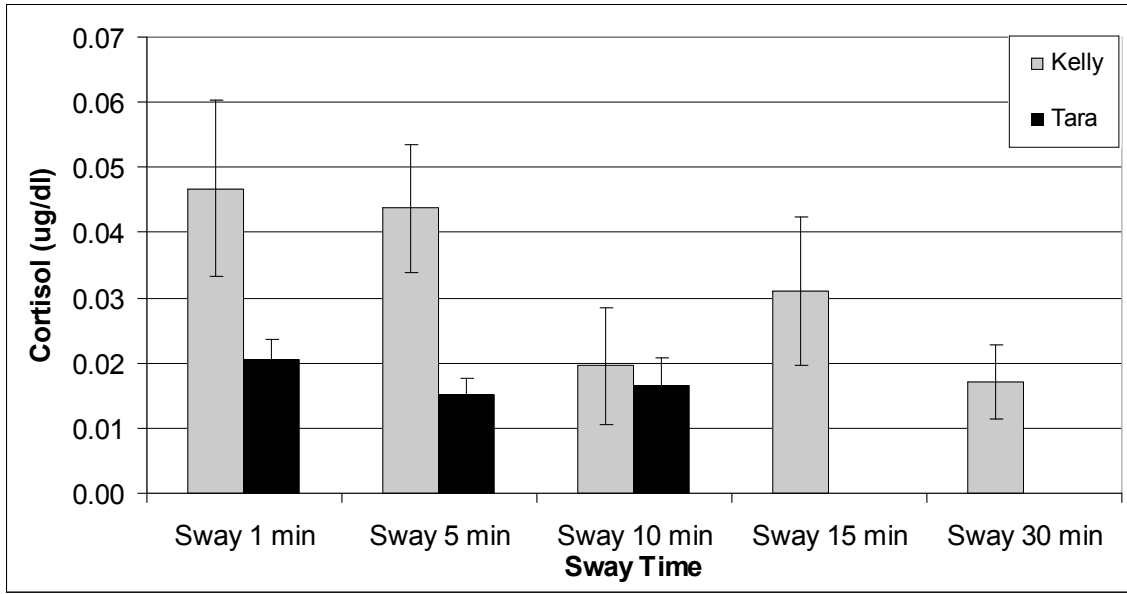


Figure 8. Mean (+/- SEM) of salivary cortisol values ( $\mu\text{g/dl}$ ) after various amount of time engaged in stereotypic swaying for Kelly and Tara. See Table 2 for sample sizes.

Mild stressor: A consistent pattern was found for the placement of each elephant in the isolation stall with its dominant conspecific. Samples collected at the beginning and end of the fifteen minute spatial restriction were similar in value. A spike was seen in the sample collected fifteen minutes after release and the levels begin to decline by thirty minutes after release (see Table 15 and Figure 9). For Kelly the salivary cortisol values were 0.048, 0.035, 0.178, and 0.069  $\mu\text{g}/\text{dl}$ . For Dottie, the values were 0.017, 0.007, 0.033, and 0.019  $\mu\text{g}/\text{dl}$ . For Tara, these values were 0.003, 0.004, 0.058, and 0.038  $\mu\text{g}/\text{dl}$ . The peak values were outside of the confidence intervals for the overall means for Kelly and Tara, but not for Dottie. This result is somewhat expected given the dominance pattern for these three elephants. Tara and Dottie have a closer relationship and Tara does not exhibit the same dominance behaviors more typical of Kelly and Dottie. Therefore, Dottie is likely to be less stressed by being isolated with Tara.

Table 15. Salivary cortisol values (in  $\mu\text{g}/\text{dl}$ ) for mild stressor in which elephants were placed in isolation with their dominant.

Elephant	Time in Isolation		Time after release	
	Beginning	15 minutes after initiation	15 minutes after release	30 minutes after release
Kelly	0.048	0.035	0.178	0.069
Dottie	0.017	0.007	0.033	0.019
Tara	0.003	0.004	0.058	0.038

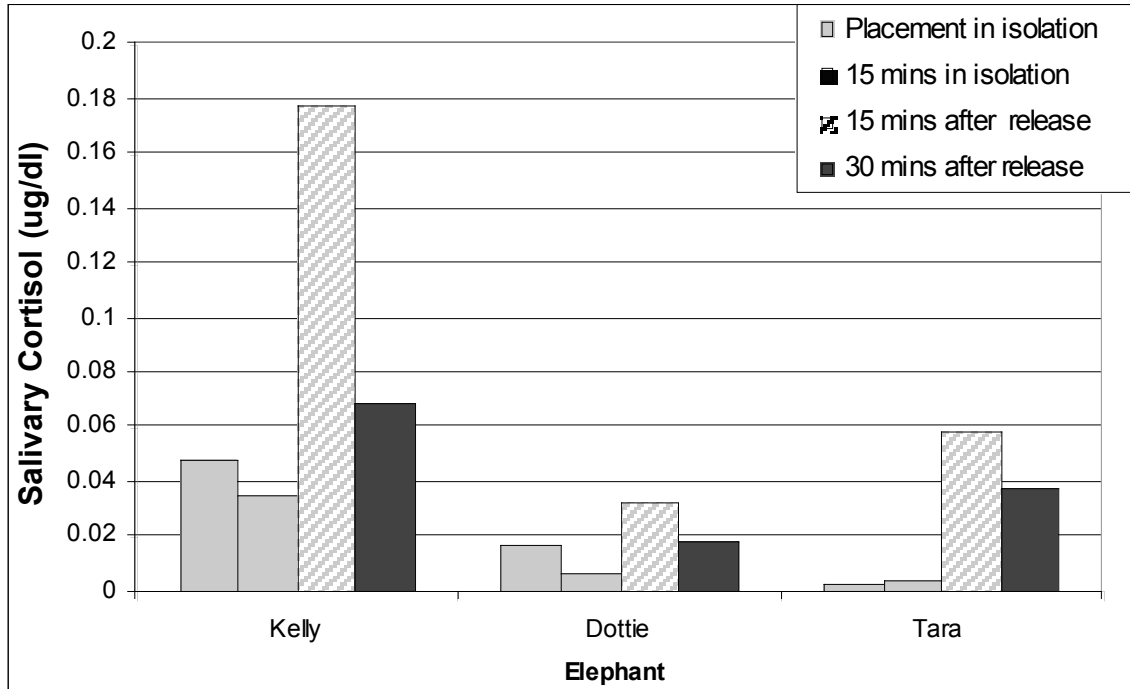


Figure 9. Salivary cortisol ( $\mu\text{g}/\text{dl}$ ), by elephant, upon placement in isolation stall with dominant a single time, after fifteen minutes in isolation stall upon which the elephant was released, fifteen minutes after release, and thirty minutes after release.

The ad lib samples taken after aggressive or potentially stressful events were mixed. The samples after the aggressive events were not outside of the confidence intervals for the means. For Tara, the sample values were 0.021 and 0.015  $\mu\text{g}/\text{dl}$  at five and ten minutes, respectively, after being struck. For Kelly, the value was 0.027  $\mu\text{g}/\text{dl}$  five minutes after being struck. Perhaps the aggression did not lead to an increase in cortisol or perhaps there were intervening events that reduced cortisol levels. However, elevated cortisol levels were found after Dottie was chained for artificial insemination training (0.054  $\mu\text{g}/\text{dl}$ ) and when Tara became agitated when she was alone in the yard, running and throwing sticks (0.049  $\mu\text{g}/\text{dl}$ ). Therefore, salivary cortisol measures do respond to rapid stress events, such as chaining and separation.

The salivary cortisol means for Kelly were not different when she was housed socially ( $\bar{x}=0.0748$ , std dev=0.0414) than when she was isolated overnight ( $\bar{x}=0.0508$ , std dev=0.0278;  $U=6.00$ ,  $p=.175$ ). Tara's means showed the opposite pattern, her salivary cortisol was actually higher when Kelly was housed separately ( $\bar{x}=0.037$ , std dev=0.0159) than when Kelly was housed with Tara and Dottie ( $\bar{x}=0.0332$ , std dev=0.0164). These differences were also not significant ( $U=11.5$ ,  $p=.834$ ). However, salivary cortisol may not track all of the fluctuations that may occur in overnight social and solitary housing.

Behavior and Cortisol: The only behavioral characteristic that has the same pattern across elephants as salivary cortisol levels is amount of time spent consuming. Tara, who consumed the most, had the lowest cortisol. Dottie had an intermediate level of consuming and cortisol. Kelly, who had the highest cortisol level, engaged in consuming the least. None of the other behavioral elements tracked the salivary cortisol

levels. All of the elephants had non-significant differences in levels of non-stereotypic activity. The two elephants that sway, Kelly and Tara, had the highest and lowest salivary cortisol values. In addition, Kelly and Tara were also the two elephants on the receiving end of the most aggressive behaviors. Kelly was the elephant struck, driven, and displaced the most. Tara followed with intermediate levels of all three occurrences. Dottie was never struck, but was driven and displaced, just less than Tara. Therefore, the only behavioral element that matches cortisol values is amount of time spent consuming.

## DISCUSSION

Overall, this study has many potential implications for the management of elephants in captivity. It provides insight into the aspects of psychological well-being (Hediger, 1964) of captive elephants in order to elucidate the factors influencing their behavior, including stereotypic behavior. Additionally, this study provided evidence for, and potential limitations of, using the combination of cortisol and behavior as a means to quantify animal welfare. Research of this type is a critical endeavor if we are to adequately exhibit these magnificent animals in captivity.

### *Serum and Saliva*

An important contribution of this study was to validate the measurement of cortisol in saliva for elephants. The cortisol assay used must be validated for any new species (Touma and Palme, 2005; Harper and Austad, 2000; Wasser et al. 2000), and full validation has not been undertaken for salivary cortisol in elephants. Because ACTH challenges were not possible with these animals, evidence from the significant correlation between plasma and salivary levels of cortisol in this species was used to indicate that the assay system is valid. Additional evidence for the precision of the tests was provided by

the intra-assay coefficient of variation and serial dilution curves. Therefore, salivary cortisol is a valid measure of cortisol in elephants allowing for inclusion in many future studies. Supplementary support can be drawn from biological validity shown via the samples taken after known stressful events (Touma and Palme, 2005). There were a few samples that provided this sort of evidence, namely the spike after isolation with a dominant, Tara's high value after exhibiting agitated behavior, and Dottie's high value after chaining. However these samples were very limited, and thus the conclusions from them are necessarily limited and can be viewed as only supplementary support.

A predictive regression equation for salivary cortisol values across the day was not discovered. However, the salivary cortisol values did tend to decrease across the day, indicating that a downward circadian trend does exist. Perhaps a study examining salivary cortisol in elephants over 24 hours or taken more frequently would discover a clearer circadian rhythm. The indistinct diurnal pattern seen in this study, mainly a lowered cortisol level in the pm, was found in other species that are provided with food throughout the day, including cows (Wagner and Oxenreider, 1972) and horses (Irvine and Alexander, 1994). Both of these studies used jugular bleeding every 15-30 minutes for 24 hours. However, a study by Alila-Johansson, Eriksson, Soveri, and Laakso (2003) collected blood from goats every two hours and found no significant daily cortisol rhythm. For the current study, a decrease across the day and between am and pm cortisol samples was found, warranting caution for future studies of elephant cortisol. Samples should either be collected at consistent times or across the day to establish a baseline trend for the individual.

## *Welfare*

While it was essential to establish salivary cortisol as a valid measure before using it to explore other avenues, of greater consequence are the conclusions related to elephant welfare. Stress is difficult to measure, and various measures may have conflicting interpretations for welfare (Mason and Mendl, 1993). Animals can exhibit a stress response, in terms of increased cortisol levels, when excited in positive situations, such as novel training, making the link between cortisol levels and welfare hard to interpret. As stated in Owen, Swaisgood, Czekala, Steinman, and Lindburg (2004), “The relationship between stress, behavior, hormones, and well-being is admittedly complex and controversial” (p. 149). Therefore, results can be complicated to interpret, but this study provides insight on this issue by examining the relationship between cortisol and behavior.

Training appears to have different effects based on level of cognitive challenge. Samples collected when the subjects were trained on a novel behavior had higher cortisol values than samples collected during maintenance training, using only established behaviors. This result provides potential evidence that increased cortisol is not always because of negative experiences. Some increases in cortisol may be beneficial (Moodle and Chamove, 1990). This claim is salient in examination events such as novel training that are likely challenging to the elephants, but not generally thought to be detrimental to welfare. This claim is based on the fact that these elephants, housed using protected contact, chose whether or not to participate in training. If it were too stressful, they would likely refuse to participate, as they frequently refuse to exercise, even for positive reinforcement (Kelling, personal observation). The value of training has been highlighted

in studies that found that animals would work for food, even when identical free food was available (Inglis, Forkman, and Lazarus, 1997; (Inglis and Ferguson, 1985; Neuringer, 1969), suggesting cognitive challenges may be a need for captive animals. Additionally, the same pattern of increased cortisol in response to novel training was seen in all three elephants, despite having a different behavior for Kelly, and no way to determine if the cognitive challenge was equal for the three elephants. Therefore, the goal for keeping elephants in captivity should not be to eliminate all sources of stress, just those that may be detrimental to welfare, either physiologically or psychologically.

The conclusion that some increases in cortisol may be advantageous for captive animals also may apply to the somewhat discrepant results found for the enrichment samples. Taken as a whole, enrichment values were not different from individual means. However, the frequency of Kelly's highest salivary cortisol values being during enrichment trials suggests that enrichment may be stressful at times. This stress may be related to the anecdotal reports of Kelly being struck or displaced when attempting to obtain the highly desirable fruit provided in the feeders or could reflect an excitement over the provided enrichment. Therefore, additional studies that collect behavioral data during enrichment trials and collect salivary cortisol after those that appeared to be more stressful for Kelly, mainly those that involved aggressive acts, would be constructive. These results support the conclusion that enrichment should not simply be provided and assumed to function as intended (Swaigood and Shepherdson, 2005). Additionally, cortisol should be examined in other cases which may lead to increases. For example, enrichment sessions that may be more exciting, such as when it has been a long time since a certain type of enrichment has been provided or when a novel form of enrichment

is used. Increases in cortisol seen after either one of these situations would not be considered detrimental to welfare, continuing to highlight the need to further detail connections among behavior, cortisol, and welfare.

Cortisol levels have been found to be inconclusive in other studies as well. For instance, in Beattie, O'Connell, Kilpatrick, and Moss (2000), pigs raised in enriched environments had higher baseline cortisol levels and higher reactions to novelty. However, adrenal weights were greater in pigs raised in barren environments, suggesting that the stress of barren environments may have overtaxed the hypothalamus-pituitary-adrenal and disabled the ability to react to acute stress in these pigs. Therefore, the pigs thought to be more stressed had lower cortisol levels. For the elephants in the current study, their cortisol values were within the reasonable range of reported amounts found in other studies of elephant cortisol. The serum cortisol values of all three elephants (Kelly: 2.734  $\mu\text{g}/\text{dl}$ , Dottie: 1.384  $\mu\text{g}/\text{dl}$ , and Tara: 1.241  $\mu\text{g}/\text{dl}$ ) were within the range of those found by Brown, Walker, and Moeller (2004), who report serum cortisol for female African elephants between 0.405 and 11.091  $\mu\text{g}/\text{dl}$  and Bettinger, Larry, Goldstein, and Laudenslager (1997), who reported a range of 0.1 to 6.75  $\mu\text{g}/\text{dl}$  for two female Asian elephants. The serum cortisol values from the current study are also within the range or lower than the baseline values reported by Stead, Meltzer, and Palme (2000) for African elephants, which ranged from 2.65 to 4.75  $\mu\text{g}/\text{dl}$ . The saliva cortisol values from this study, ranging from 0.003 to 0.26  $\mu\text{g}/\text{dl}$ , are also reasonable based on the two previous studies. Bettinger et al. reported that most salivary cortisol values for the two Asian elephants were below 0.1  $\mu\text{g}/\text{dl}$ , which was below the sensitivity of the test at the time. The means found in the current study of 0.054 , 0.034, and 0.030  $\mu\text{g}/\text{dl}$  are lower than the

salivary cortisol means reported for Asian elephants in Dathe, Kuckelkorn, and Minnermann (1992). Their reported mean values ranged from 0.223 to 1.15  $\mu\text{g}/\text{dl}$ . Therefore, the elephants in the current subject do not have cortisol means outside of reasonable values, meaning that their welfare should not be questioned based on their basal cortisol values. Additionally, these subjects did not have blunted cortisol responses to the mild stressor when each elephant was placed in the isolation stall with its dominant and rises in cortisol also tracked agitated behavior and training for artificial insemination training.

Although the cortisol values were within previously reported ranges, the values of serum cortisol found in this study were higher than those found by Wilson, Bloomsmith, and Maple (2004). However, the trends were the same. In Wilson, et al., Kelly had the highest mean serum cortisol value of 1.0  $\mu\text{g}/\text{dL}$ , followed by Dottie at 0.8, and then Tara at 0.6. In this study, the same pattern was observed, with values highest for Kelly 2.734, next highest for Dottie 1.384, and lowest for Tara 1.241. It is unclear what may have led to an increase in serum cortisol, but one difference is that the samples in Wilson et al. were all taken in the afternoon, consistently on Thursday afternoons between 1300h and 1500h, whereas the samples for the current study were taken across the day. Given that cortisol values were found to decrease across the day, this difference may explain at least a portion of the increase. However, even the pm only samples, which had lower mean cortisol values than total serum, were higher in the current study than Wilson et al. An additional factor may be changes in husbandry that may have occurred with the time lag between the studies, with the most significant change being the conversion from free contact to protected contact. It is unclear how this sort of change may affect the animals

involved, however the current data provide support that cortisol does not rise to unreasonable levels by the removal of the human portion of the elephant's social group. An additional factor which may explain Kelly's increased cortisol is that she contacted Leptospirosis, a bacterial illness that may have affected her cortisol levels (see Plank and Dean, 2000 for a discussion of Leptospirosis effects in humans).

A main focus of the current study was how stereotypic swaying affects cortisol values. Swaying decreases cortisol values, suggesting that it functions to deal with excess stimulation. This conclusion has huge implications for the debate that rages on the possible functions of stereotypic behavior and the relationship between stereotypic behavior and cortisol. High rates of stereotypic behavior exhibited daily by an individual may suggest decreased welfare because the animal is displaying a need to decrease excess cortisol. Consequently, managers may still want to decrease stereotypic behavior, but attempts to decrease this behavior should address the excess stimulation itself and not simply prevent stereotypic behavior. Cortisol reduction through stereotypic behavior seems to be larger for Kelly than Tara based on visual inspection and the fact that Tara's swaying values do not differ from the individual means that were calculated using only the non-swaying afternoon samples. Most likely this behavior serves a different function for each elephant. This conclusion is further supported by the fact that Tara's swaying pattern includes pauses, is more energetic, and she because sways for shorter duration. Supplementary studies should be performed to further examine this disparity and advance the discussion of the function of stereotypic behavior. Repeating the baseline and swaying cortisol values with other elephants would be beneficial. Additionally, given the potential connection, more samples can be collected from these particular animals to

further investigate potential differences in swaying between Kelly and Tara and how swaying affects cortisol.

### *Behavioral Data*

This study also provided a great deal of information from the behavioral data, including a quantification of dominance and social relationships. Although better known for their cooperative behavior, female elephants in the wild also demonstrate aggressive interactions, despite being closely related (Dublin, 1983). These aggressive acts tend to relate to dominance position or competition for resources (Douglas-Hamilton, 1973). Aggression is thought to establish social roles in the artificial family groups created in captivity (Adams and Berg, 1980), which are more flexible than the kin based groups of wild elephants (Garaï, 1992). Dominance and direction of aggression appear to be well established in these elephants. The anecdotally reported circular dominance was confirmed based on observed aggressive acts. Dottie was dominant to Kelly who was dominant to Tara who was dominant to Dottie. Dottie and Tara also appeared to have a closer relationship than either had with Kelly. This difference was confirmed through observations of affiliative behavior and time spent proximate to another elephant. Overall, levels of aggression were fairly low, with only five incidents of one elephant striking another in 90 hours of observation. Although not all of those hours involved social situations for the entire or even any of the observation, the majority of the observations did include the option for social interaction. Additionally, affiliative behaviors, namely trunk touching, occurred more frequently than aggression. Trunk touching was observed a total of 21 times, with the behavior being more frequent between Dottie and Tara who were observed to trunk touch 16 of the 21 times. Dottie

and Tara were also found to be proximate to another elephant a greater proportion of time than Kelly was. The behavioral data, especially the affiliative behaviors and propensity for Dottie and Tara to remain proximate, provide additional support that captive elephants are social animals and they form strong social bonds, despite being unrelated and housed in unnatural groupings (Gruber, Friend, Gardner, Packard, Beaver, and Bushong, 2000; Garai, 1992; Adams and Berg, 1980).

Behavioral data also provided an estimation of how these animals tend to spend their time. Tara and Dottie spend the vast majority of their time consuming food. Kelly's time spent consuming is much lower, perhaps because she devotes approximately a quarter of her time on exhibit to swaying. Therefore, only Dottie and Tara had time budgets that can be considered similar to those found in wild elephants (Wyatt and Eltringham, 1974). Kelly also was observed to sway a much larger amount of time than the 1% reported during the non-shift times in Wilson, Bloomsmith, and Maple (2004). There was a difference in the methods of data collection between the present study and Wilson et al. The data in this study were collected using Observer software, allowing for true determination of percent duration, whereas the data collected by Wilson et al. were collected using instantaneous focal samples of behavior taken every 30 seconds, providing the percentage of scans in which the behavior of interest occurred. However, such a large difference is more likely because of an actual difference in percent of time spent swaying, a result not too surprising given the time difference in data collection. The behavioral data from Wilson et al. were collected in late 2000 and early 2001, almost seven years before the current data. As mentioned before, the management of these elephants has been changed from free to protected contact. The effects of this change

whose effects are uncertain and should be further explored. Given recent changes in elephant management which will produce a generation of elephants that have never been chained, it will be useful to study these unchained individuals and see if the percentage of elephants that sway is smaller than in those who were chained. In addition, many of the staff have changed since the previous study. Kelly's illness may also be a factor, as she was isolated while ill, approximately two months during the day and six months at night, and may have increased swaying because of her isolation. Other species, mainly primate, have shown increased stereotypic behavior in response to increased time spent in single cage housing (Lutz, Well, and Novak, 2003; Bellanca and Crockett, 2002). Additionally, increased rates of stereotypic behavior may aid in coping with environmental stress. Thus, animals may be under chronic stress, but have lowered levels of cortisol because of these coping behaviors, such as stereotypic pacing or swaying (Broom and Johnson, 2003). However, individuals exhibiting high levels of stereotypic behavior cannot be labeled as highly stressed without additional evidence. Kelly's stereotypic swaying rate is higher than when previously measured, but is not as high as other reports (Elzanowski and Sergiel, 2006) and her salivary cortisol values are within normal ranges, thus neither line of evidence suggests severely reduced welfare. Although the increased level does warrant additional research, perhaps into avenues such as predictability or providing behavioral alternatives to reduce her need to sway.

Kelly's increased stereotypic swaying may also be a factor of age. A contradiction exists between studies of elephants that have found both an increase and decrease in stereotypic behavior in elephants based on age (Gruber, Friend, Gardner, Packard, Beaver, and Bushong, 2000; Wilson, Bashaw, Fountain, Kieschick, and Maple,

2006). Other studies with various species have also disagreed on the effects of age on stereotypic behavior. For instance, Vickery and Mason (2003) found that older bears with increased time spent in captivity performed higher rates of stereotypic behavior. This same result was found in mink (Mason, 1993). Because the elephants in the current study have now been the subjects in several research projects that have recorded the frequency of stereotypic behavior, it will be useful to continue studying these animals to observe any changes in stereotypic behavior rates, especially after their management style has been consistent for a few years.

### *Cortisol and Behavior*

Cortisol levels have been correlated with dominance level in many species. Although Bercovitch and Clarke (1995) found no significant difference between levels of cortisol in low and high ranking adolescent male rhesus macaques, they did find that low ranking males had more variable cortisol concentrations. Other studies have found significant differences, but not always in the same direction. Creel, Creel, and Monfort (1996) state that in the wild, dominants often have higher cortisol levels, perhaps because they have to be aggressive to maintain social status. They suggest that highly aggressive dominants may be more stressed in the wild, but in captivity subordinates cannot avoid aggressive dominants and thus may have elevated cortisol levels. Abbott et al. (2003) performed a meta-analysis of different captive primate species and found that, although in a few species subordinates had lower cortisol levels than dominants; generally subordinates had higher cortisol levels. Subordinates tend to have higher cortisol levels in species in which being a subordinate goes along with higher rates of stressors, both physical and psychological, and fewer sources of social support, which occurs mainly in

species in which subordinates are not closely related to other members of the social group. In this group of elephants, dominance is circular; therefore there is no true subordinate. However, Kelly, who has the highest and most variable cortisol values, is also the target of the most aggressive behaviors and appears to have the least social support. On the other hand, Tara received an intermediate rate of strikes and displacements, but was found to have the lowest cortisol. Dottie, who was never struck, had the second highest cortisol values. Cortisol may be elevated only with a certain level of aggression. Some or all elephants able to cope with lower levels such as those Tara received, or elephant cortisol values may be unrelated to dominance or mediated by social support. Further research to establish baseline cortisol values in additional elephants may help clarify the dominance status and cortisol link in captive elephants.

#### *Future Research*

Several possible future studies have been mentioned; the most critical being research to further examine welfare in elephants. The current work has provided an excellent framework to further build upon. As such, a great deal of work is suggested by the results of this study. It would be useful to repeat many aspects of this study with additional elephants, and perhaps even additional species that engage in stereotypic behavior to expand the scope of results and generalizability. Of course, baseline values would have to be established for any additional subject. Additional baseline cortisol analyses would allow for an expansion of the exploration into the circadian rhythms of elephant cortisol and the connection between cortisol and behavior, looking for additional trends with stereotypic rate, percent of time spent consuming food, and any dominance or aggression links with cortisol. Other studies should also examine the cases in which

increased cortisol occurred after positive events, such as novel training and enrichment to examine the effects of these husbandry events. The enrichment used in this study remained uniform to ensure high levels of interaction and consistency. Future studies examining the effects of novel enrichment would be beneficial given the difference seen between maintenance and novel training. Enrichment studies integrating cortisol and behavior data would also be useful to determine if the increases seen in many of Kelly's enrichment samples were because of excitement over the enrichment or incidents of aggression.

More studies should also be performed to further analyze the potential function of stereotypic behavior, given the differences between Kelly and Tara. Future studies could scrutinize cortisol reduction differences between elephants with differing swaying patterns and rates, to confirm the difference seen here between an animal for whom swaying seems obligatory and thus has a greater coping function and one for whom swaying is much less frequent and had less of a coping effect. Additionally, studies could examine the effects of any management changes intended to reduce stereotypic swaying rates to determine how they affect the coping function of stereotypic behavior. Stereotypic behavior may indicate past and not current sub-optimal conditions (Hediger, 1964). In some cases, stereotypic behaviors are not thought to relate to stress at all. For instance, Mason and Latham (2004) state that "normal human stereotypies, such as thumb-sucking and gum-chewing, are not generally associated with stress" (p. S59). One possible solution is to turn to other data, such as conducting preference tests giving the animal choices instead of forcing them into certain situations. For example, with the subjects of this study, Kelly could be given the choice of being solitary or remaining with

her social group overnight. Control is often lacking in captivity, which is thought to lead to reduced welfare. Indeed, research has demonstrated that animals have a preference for control (see Bassett and Buchanan-Smith, 2007). These preferences demonstrated by captive animals may indicate needs of the animals and if they are denied, may result in reduced welfare (Veasey, 2006). Nevertheless, the harder an animal is willing to work indicates a greater preference and is more likely to indicate a resource or opportunity without which the animal is likely to suffer (Cooper, 2004; Dawkins, 1983). For example, elephants will forfeit opportunities to feed for a chance to be near confined members of their social group, suggesting that social contact is preferred and may be especially important for the welfare of this species (Veasey, 2006). Additional studies of choice and cortisol levels would be informative.

Other future studies, potentially not involving cortisol, should also be performed to elucidate the role of stereotypic behavior and determine if there are behavioral differences between elephants for who swaying is obligatory and those who engage swaying only infrequently. One possible study is to examine attentiveness to environment during swaying in a method similar to the study performed with polar bears (Weschler, 1992). In the Weschler study, polar bears were found to pause for olfactory investigation of novel scents, even when pacing. Also, stereotypic behavior has been positively correlated with behavioral persistence, examined through an extinction task, in bank voles (Garner and Mason, 2002) and Asiatic black and Malayan sun bears (Vickery and Mason, 2003). These results suggest that there may be a difference in animals that perform stereotypies; perhaps they have lost a degree of behavioral flexibility. Further research with additional species, including elephants, would be useful to gain additional

understanding of how stereotypic behavior affects behavioral flexibility. Additionally, studies such as these would allow for further comparisons between Tara and Kelly to gain additional insight into differences in the function of their stereotypic behavior.

Given the evidence that stereotypic behavior may function to reduce excess arousal and the finding from Wilson, Bloomsmith, and Maple (2004) that Kelly swayed considerably more before the elephants were shifted into the barn at night, studies examining the effects of predictability on elephant behavior may be warranted. Predictable husbandry events may be stressful for these animals, causing excess stimulation, especially in Kelly, creating excess stimulation. Perhaps these events have inconsistent signals, meaning for instance that food related sounds may begin variable durations before food delivery (Waite and Buchanan-Smith, 2001). Chimpanzees were found to exhibit higher inactivity and rates of abnormal behavior before feeding when on a predictable schedule than an unpredictable one (Bloomsmith and Lambeth, 1995). Therefore, unpredictable schedules may be beneficial for some zoo animals. It would be interesting to examine how an unpredictable shift schedule or a precise signal to indicate when shifting would occur would change the stereotypic behavior of these animals. Animals frequently chose predictability over unpredictability for aversive events and occasionally for positive events, such as feeding. It is thought that predictability or at least reliable signaling allows animals to prepare for the event, such as salivating when food delivery is signaled (Bassett and Buchanan-Smith, 2007). However, predictable feeding schedules often lead to anticipatory activity, mainly locomotor stereotypies. Additionally, environments that are highly predictable may be stressful because animals are adapted to deal with the variation of nature, thus too much predictability may become

boring (Bassett and Buchanan-Smith, 2007). Predictability may have other drawbacks. For instance, Bloomsmith and Lambeth (1995) found an increase in species-typical behaviors, and thus potentially welfare, when chimpanzees were fed on an unpredictable schedule, which could suggest that predictability may be more stressful if the animal has no control. However, the removal of predictability can be concerning because its loss, especially loss of a sense of control, may lead to stress, frustration and/or aggression (Bassett and Buchanan-Smith, 2007), thus removal of predictability should be investigated.

Another avenue related to predictability to investigate is the addition of a signal to indicate when shifting into the barn and access to food will occur, which could be beneficial for these animals. However, it is crucial to remove all unreliable signals (Bassett and Buchanan-Smith, 2007). Additionally, perhaps some form of enrichment could be provided prior to shifting, allowing them a behavioral outlet or at least some distraction to occupy them while they have to wait. This enrichment provision could decrease the excess arousal and thus the behavioral need to engage in stereotypic behavior. Another option is providing the elephants with choice. Allowing giant pandas to choose their location led to lower urinary cortisol levels and less time engaged in agitated behaviors, which included pacing, scratching, and door-directed behavior (Owen, Swaisgood, Czekala, and Lindburg, 2005). Perhaps these elephants, who are often allowed to choose their location at night, have decreased cortisol and stereotypic behavior when allowed this choice. Determining the benefits of this choice would be useful, especially in understanding the effects on the elephant when this choice is not available, mainly when it is too cold for the elephants to be given outdoor access.

Several other avenues deserve investigation. Tara's increased salivary cortisol when Dottie and Kelly were in the isolation stall for Kelly's mild stressor, provides physiological evidence that even short term separations can be stressful, confirming behavioral evidence found in Garaï (1992), and should be explored further. Additionally, the link between elevated cortisol and decreased fitness, mainly absence of cycling, should be explored in more elephants. Brown, Walker, and Moeller (2004) found that there were no significant differences in cortisol levels based on estrous cycle status or stage, or species. They did find that of the five elephants with elevated mean cortisol, four were non-cycling, but these four represented only a small portion of the non-cycling females. Therefore, the link between cortisol and reproductive problems should be further examined, especially if reproductive problems are exhibited by a previously cycling female. Of the elephants in the current study, Kelly does not cycle and she does indeed have the highest cortisol. Tara cycled, but only sporadically. Dottie cycled and became pregnant by artificial insemination.

Additional work should also examine the effect of management styles on cortisol. Given the management changes from free to protected contact in approximately half of AZA accredited zoos (Hancocks, 2008), more work needs to be done to examine the effects of this transition on elephants. Further work should also be done to examine the development of stereotypic behavior, scrutinizing the differences between elephants that were never chained and those that were chained for years, and how it changes, either in rate or form, with age. The elephants in the current study would be ideal subjects for a follow-up study to determine if swaying changes with age as they have been studied at several points in time.

### *Limitations*

One potential problem of the current study is that the data were collected throughout the year. Foley, Papageorge, and Wasser (2001) found elevated fecal cortisol levels in free-ranging African elephants during the dry season, inversely correlated to rainfall, suggesting that there may be a difference in elephant cortisol based on season. However, this increase may have been related to the stress of trying to survive, as limited rain would require more work to find food, such as traveling greater distances. Brown, Walker, and Moeller (2004) found no significant differences in serum cortisol in captive African and Asian elephants related to season, thus season is most likely not a factor in cortisol fluctuations in captive elephants.

Another obvious issue with the current study is the small sample size. This study was exploratory, and was limited by the availability of subjects and restricted by costs of the cortisol assays. Any findings are unavoidably limited in generalizability because of the small sample size, but any trends found across all subjects suggest aspects of the study that should be repeated with other elephants, allowing a better focus and more sensible use of resources. Additionally, the data collected are still informative and the small sample size is fairly compensated for by calculating statistics on individual animals separately. Swaisgood and Shepherdson (2005) state that this method is “less than ideal,” but is considered “a relatively legitimate approach to dealing with small sample sizes” with the caveat that generalization to the entire captive population is limited (p. 508). Additionally, any significant results found in only three elephants are likely to be very powerful differences and indeed there were significant results in which the same pattern was seen even in these three elephants that vary on mean cortisol level and stereotypic

swaying amounts. Additionally, the exploratory nature of this work generates a needed framework for further research utilizing salivary cortisol.

### *Conclusions*

1. Salivary cortisol is a valid measure in elephants and can be used, in conjunction with behavior, to investigate elephant welfare.
2. Elephants did not exhibit a distinct circadian rhythm of salivary cortisol, although cortisol levels did decrease across the day.
3. Stereotypic behavior varied both qualitatively and quantitatively between elephants. Stereotypic behavior was also found to reduce cortisol levels, suggesting that it may have a function.
4. Novel training led to an increase in salivary cortisol compared to maintenance training, warranting the conclusion that novel training is a cognitive challenge for elephants.
5. Cortisol after enrichment did not differ from overall means. However many high cortisol values were observed after enrichment, suggesting that enrichment may be either exciting or stressful because on competition.

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