ABSTRACT

Title of Document:	BLAZE: BETTERING THE LIVES OF ANIMALS IN ZOO ENVIRONMENTS ENVIRONMENTAL ENRICHMENT AND ITS EFFECTS ON STRESS HORMONES IN CAPTIVE FELIDS Logan Anbinder, Amelia Cordell, Gretchen Downey, Kelly Freudenberger, Shabaab Kamal, Thao Khuc, Joshua Lacey, Caitlin Moore, Emmarie Myers, Andrea Schmidt Gemstone Program, 2012
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Captivity can induce high levels of stress in zoo animals, leading to health and behavioral problems that hamper conservation efforts, reduce the effectiveness of education, and negatively affect animal welfare. Zoos employ environmental enrichment to mitigate stress, but the effectiveness of various types of enrichment is poorly understood. We surveyed enrichment practices at 39 zoos nationwide and then used noninvasive fecal hormone analyses to monitor stress in three species of felids under different enrichment programs at two zoos. Baseline analyses at the National Zoological Park showed individual differences in stress hormone levels but no seasonal effects. Contrary to expectations, a novel enrichment program at Plumpton Park Zoo produced higher cortisol levels than a reduced enrichment program. Results suggest that novel objects that elicit active engagement may cause transient increases in stress hormones. Further long-term study is needed to elucidate whether this has a positive or negative effect on well-being.

BLAZE: BETTERING THE LIVES OF ANIMALS IN ZOO ENVIRONMENTS

ENVIRONMENTAL ENRICHMENT AND ITS EFFECTS ON STRESS HORMONES IN CAPTIVE FELIDS

By

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Chapter 1: Introduction

1.1 Overview

In the past decade, research increasingly has shown the critical importance of biodiversity in maintaining a functioning complex ecosystem, a relationship that was largely underestimated in the past (Duffy 2009). However, as human population grows and as the Earth's climate changes, the Earth's biodiversity has become threatened and continues to decline (Rands et al. 2010). Now, conserving biodiversity – the variety of ecosystems, habitats, species, organisms, and genes comprising life on Earth – has become a priority of local, national, and international scale (United Nations 2007; Rands et al. 2010).

The Earth's ecosystems provide us with many products and services, such as material goods from natural resources (medicines, timber, food), environmental functions (flood protection, climate control), and other benefits (Rands et al. 2010). Because humans are dependent on these ecological services, it is imperative to protect the Earth's diverse ecosystems and their biodiversity to ensure human survival, as well as the survival of the other organisms inhabiting Earth. The United Nations recognized the importance of biodiversity for the world and ratified the UN's Convention on Biological Diversity in 1992 (United Nations 1992). Additionally, conserving biodiversity by reducing the rate of biodiversity loss was made one of the UN Millennium Development Goals, and the UN declared 2010 as the International Year of Biodiversity (United Nations 2007; Rands et al. 2010).

International attention has led to increased conservation organizations and efforts, yet trends show a continuing decline of biodiversity (Rands et al. 2010). Species, habitats, and resources are continuing to suffer. According to the International Union for Conservation of Nature (IUCN), the extinction of species is currently occurring much faster than it would in the absence of human effects, up to four magnitudes as fast as a result of pollution, exploitation of resources and species, degradation and destruction of habitats, climate change, and invasive non-native species (IUCN 2012; Rands et al. 2010). The IUCN's Red List now lists 19,570 species as threatened, vulnerable, endangered, critically endangered or extinct, although only 4% of the known species in the word have been evaluated. The Red List includes 7,108 species of vertebrates, 3,297 species of invertebrates, and 9,156 species of plants. Within vertebrates, 1138 mammalian species are threatened, and 86% of these species are threatened due to habitat loss (IUCN 2012).

According to experts, threatened species have a high probability of extinction in the coming decades (Chivian 2003). As a result, zoos have increasingly worked to promote species and habitat persistence, as well as maintain genetic diversity. Although all aspects of biodiversity are important, preventing species extinction is critical because it is irreversible. As the loss of genetic variability increases, the viability of diverse species populations decreases, ultimately leading to extinction (Laikre 2010). The purpose of zoos has shifted to encompass these conservation efforts, as well as research and education of the public to further preservation of wildlife and biodiversity (Lewis 2010; Conway 2011).

Even with the increased global efforts to support conservation, the general public still lacks knowledge about the importance of biodiversity, the conditions facing many species and habitats, and the steps they can take to reverse these trends (Patrick et al.

2010). By seeing living animals in simulated representations of their native habitats, visitors can gain a better understanding of, and appreciation for, the natural world (Routman et al. 2010). Zoos have a unique ability to provide this knowledge to increase public awareness, while preserving biodiversity through captive breeding, reintroduction programs, and *in situ* conservation projects. In the United States alone, approximately 140 million people visit zoos accredited by the Association of Zoos and Aquariums (AZA), which account for only 10% of the USDA licensed zoos in the country (Routman et al. 2010; AZA 2009). With 600 million zoo attendees worldwide each year, well-run zoos have the ability to foster positive attitudes and provide visitors with a direct connection to wildlife (Zimmerman 2010). In 2007, the AZA stated, “We envision a world where all people respect, value, and conserve animals and nature” (Routman et al. 2010). To achieve this, zoos must have psychologically and physiologically healthy animals for their conservation efforts. Although many zoos focus on conservation efforts and optimizing the welfare of their animals, some critics of zoos believe that animals should not be housed in captivity, regardless of the intent of the zoo community (Kreger and Hutchins 2010).

Despite the best attempts of the zoo community, animals’ enclosures will never be true representations of their natural habitats because they are small and synthetic exhibits. As a result, animals sometimes use coping mechanisms known as stereotypies (or abnormal repetitive behaviors) to relieve stress induced by the captive environment (Pitsko 2003). In addition to causing aberrant behavior, stress may cause reduced fecundity and can be detrimental to animals’ mental and physical health (Van Metter et al. 2008). On the other hand, when animals’ stress levels are normal, the animals have improved resistance to disease, improved fecundity, and overall better health, which enhances their well-being. Additionally, physiologically and psychologically healthy animals provide researchers and the public with more "natural" representations of what the animals' lives would be like in the wild. Furthermore, preservation of these natural behaviors is important for those zoo animals that are destined to be released into the wild (Mellen and MacPhee 2001). Solving stress-related problems in captive animals is thus a high priority of modern zoos to both protect the animals and meet fundamental zoo goals.

Captive animals provide an opportunity for the zoological community to expand its knowledge of scientific fields that span endocrinology, behavior, reproduction, and physiology. The more closely the physiology and behavior of captive animals mirrors that of their wild counterparts, the more generalizable the results of this research will be. Similarly, captive animals whose behaviors are perceived as "natural" provide a forum for education of the public. Educational programs stress the life history traits of the species being exhibited while appealing to the audience's desire to learn (Churchman 1987). Visitor education is dependent on watching the animals; therefore, more active animals expressing their species-specific behaviors will engage the visitors and increase public appreciation for wildlife (McPhee and Carlstead 2010).

Additionally, zoo visitors wish to experience the animals in as natural a setting as possible. Many zoo employees and visitors express feelings of sentiment towards captive animals and feel a particular ethical responsibility to ensure the well-being of these creatures, especially the charismatic megafauna such as big cats (Rabb 2004). Animals that do not exhibit natural behaviors are often seen as unhealthy by visitors, and visitors tend to feel that zoos are not preserving biodiversity and endangered species if the animals are unhappy (McPhee and Carlstead 2010).

Environmental enrichment has become an important part of the routine husbandry schedules in the past few decades, as the zoo community has recognized its benefits for captive animal welfare. Providing animals with environmental enrichment serves in part to elicit species' natural behaviors by increasing complexity and the opportunity for choice in the animals' environments, thus improving an animal's well-being (Van Metter et al. 2008). There are several types of enrichment, including structural, tactile or manipulative, sensory, feeding, and social interaction. Several recent studies (e.g., Bashaw et al. 2003, Skibiel et al. 2007, Moreira et al. 2007, Van Metter et al. 2008) have attempted to assess the effects of enrichment on captive animal welfare.

The effects of enrichment can be assessed using behavioral or endocrine monitoring. In terms of behavior, the efficacy of enrichment is judged based on increased expression of a variety of species-specific behaviors to more closely represent the behaviors of the animal's wild counterpart (Kagan and Veasey 2010), as well as a decrease in abnormal and stereotypic behaviors (Swaisgood and Shepherdson 2005). Glucocorticoids are hormones related to the body's stress response, and their levels in the animals' blood, urine, or feces can also be used to assess enrichment. A reduction in mean glucocorticoid levels, following introduction of enrichment is thought to be indicative of improved animal welfare (Moreira et al. 2007).

Although research regarding the effects of enrichment on animal behavior and physiology are increasing, few studies have compared the effectiveness of different types of enrichment on individual species (Van Metter et al. 2008). Species vary in their responses to their environments and in the behaviors they need to express to ensure survival. Additionally, there are interspecific differences in their responses and adaptability to the captive environment. More understanding of species' needs is critical. For zoos to achieve their conservation goals and protect our planet's biodiversity, they must first ensure the well-being of animals in captivity. For some species, zoos may be the last resort for species continually threatened by loss of habitat, overexploitation, disease, poaching, and more (Conway 2011).

1.2 Research Questions

Because felids are especially difficult to maintain in captivity (Clubb and Mason 2007), we aimed to investigate the most effective means of alleviating undue stress and encouraging natural behaviors. This knowledge will ultimately improve a zoo's ability to maintain healthy and reproductively viable populations of large felids.

Our research questions are "How does the use of enrichment vary among zoos?" and "How do specific programs of enrichment affect endocrine measures of stress?" Our research consisted of three phases in which we investigated enrichment techniques and their effects on three species of felids. In the first phase, we conducted a survey of enrichment techniques used for captive felids in 39 diverse zoos and wildlife preserves across the United States. The second phase evaluated the effects of season, day of the week, and type of enrichment on fecal cortisol levels in felids maintained at the Smithsonian National Zoological Park (NZP). In the final phase, we manipulated enrichment schedules in felids maintained at Plumpton Park Zoo (PPZ) to measure the effects of differing enrichment programs on fecal cortisol levels.

1.3 General Study Hypothesis

We hypothesized that captive lions (*Panthera leo*), tigers (*Panthera tigris*), and cougars (*Puma concolor*) under a novel, comprehensive enrichment schedule would show differences in fecal corticoid levels compared to a reduced enrichment schedule. Wielebnowski and Watters (2007) stated that these hormone levels could provide insight to an animal's physiological response to their environmental and social conditions. Because enrichment is designed to alleviate stress and elicit more natural behaviors, we expected that fecal corticoid levels would decrease under a novel enrichment schedule. We also anticipated possible differences between individuals and species in their responses to enrichment, due to physiological and temperamental differences (Van Metter et al. 2008)

1.4 Methodological Framework

The ultimate goal of our research was to examine the effects of different enrichment programs on fecal cortisol levels of captive felids. We divided our study into three phases. The first phase consisted of a survey in which we investigated enrichment practices used for felids in zoos and wildlife preserves throughout the United States. These results provided a context for our subsequent endocrine studies and showed which enrichment techniques were most commonly used and what factors were perceived as limiting the use of enrichment. We further looked for significant differences in practices and perceptions among various types of zoos (e.g. AZA vs. non-AZA accredited, public vs. private, large vs. small).

In the second phase, we investigated the endocrine responses of lions and tigers maintained on a predetermined enrichment schedule at the Smithsonian National

Zoological Park in Washington, DC, using fecal cortisol monitoring, a non-invasive technique for evaluating stress responses. We evaluated the possible effects of season, day of the week, and type of enrichment (feeding, manipulative, or sensory) on fecal cortisol levels.

Using the results obtained from the first two phases of our study, we developed a comprehensive enrichment schedule that we believed to be representative of, and feasible for, most zoos. The third phase took place at Plumpton Park Zoo in Rising Sun, Maryland. We applied three different enrichment schedules to the zoo's tiger and cougar. For nine successive weeks, we rotated enrichment schedules between weeks of reduced enrichment, the enrichment program already in use by PPZ, and the comprehensive schedule we developed. We then used fecal cortisol monitoring to compare the effects of the different enrichment schedules. Fecal cortisol assays for phases two and three were conducted by the Smithsonian Conservation Biology Institute (SCBI) in Front Royal, VA.

1.5 Research Contribution to the Field

Our research focuses on felids because they are especially susceptible to the negative effects of stress (e.g., Bashaw et al. 2007; Clubb and Mason 2007; Terio et al. 2004; Mellen and Shepherdson 1997). This vulnerability most clearly manifests itself in reduced fecundity (Terio et al. 2004). Previous research (e.g., Moreira et al. 2007) suggests that a heightened endocrine stress response is linked to decreased fecundity, and we hope that by learning how to lower stress levels of captive felids, we will be able to understand how to encourage felid reproduction in captivity more effectively (Jurke et al. 1997). Increasing felid fecundity will consequently aid zoos in their goal of conservation by preserving genetic diversity in those populations. Some individuals breed readily, while others may not breed at all. Enrichment may provide a way for zoos to facilitate reproduction in those individuals that are not breeding well. By providing subjects whose behavior closely resembles that of their wild counterparts, enrichment may further zoos' additional goals regarding research and education (Ogden and Heimlich 2009).

This research will allow for broader generalizations about the effects of specific enrichments by focusing on three felid species. By investigating the continuum of enrichment techniques in use currently and the effects of various programs of enrichment on the cortisol levels of felids, we aim to further the goals of the zoo community.

Chapter 2: Literature Review

2.1 Zoos

2.1.1 History of Zoos

What we now refer to as the "zoological park" has a long history, dating back approximately 4300 years to the first known zoo in Ur, Iraq (Hancocks 2010). Records from 1490 BCE tell of animals kept by Queen Hatshepsut in Egypt (Zimmerman 2010). Since that time, humans in many developed societies, including ancient Egypt, China, and Greece, have kept animals in captivity as a demonstration of wealth, power, and control over these animals (Hancocks 2010). The Renaissance gave rise to many private menageries, owned by the wealthy and aristocratic. Unlike modern day zoos, these menageries were private collections of exotic animals meant for only the social elite. In the 18th century, traveling menageries brought wild animals to the attention of the general public. Because most people had never seen exotic animals before, these menageries had an educational aspect, albeit a very different one from today's modern zoos. Zoos of that era allowed the public to enjoy the exotic nature of the animals while creating a feeling of human superiority and control over them.

The opening of the Zoological Garden in London in 1828 marked the shift from personal animal collections to facilities based on scientific research principles. The London Zoo was the first to be called a "zoo" by the zoo community and public (Hancocks 2010). In the late 19th century, more zoos were built throughout Europe, and almost all metropolitan areas, in countries such Australia, Japan, India, Germany, Egypt, England, and the United States (Hancocks 2010). Still, most of these facilities did little to further the welfare of their animals, most being so-called “postage stamp” collections, where animals were housed in unadorned adjacent metal cages (Ebersole 2001). These zoos did not inform visitors about the animals’ biology or natural habitats (Hancocks 2010). Zoos at this time were meant to attract visitors and allow them to experience viewing animals up close.

The 20th century brought the idea of “naturalism” to zoos worldwide, although some zoos adopted the concept more readily than others (Hancocks 2010). In 1907, the Tierpark Hagenbeck in Hamburg, Germany became the first zoo to use open enclosures and moats, rather than bars, to separate animals from each other and from visitors (Ebersole 2001). While this was done to give visitors a more realistic viewing experience, rather than to benefit the animals, it is often viewed as the first step towards current environmental enrichment practices (Rothfels 2002). Zoos in the United States during this time were modeled after the traditional zoos in Europe, and many zoo designers opposed the naturalistic approach in favor of sterile, barren enclosures that were easy to clean but did not consider the welfare and needs of the animals (Hancocks 2010). Heini Hediger published two books (1950, 1955) suggesting that zoo exhibits should be designed based on biological concepts because the exhibits of the time were not providing the captive animals with their basic needs (Kreger and Hutchins 2010).

By the 1970s, zoos had begun to design exhibits to more closely mirror an animal’s natural environment (Mellen and MacPhee 2001). The needs of the animals became a priority as understanding of animal welfare improved (Hancocks 2010). As researchers learned that a naturalistic environment had a greater educational and emotional impact on zoo visitors, even public areas of zoos were designed to mimic nature to truly surround visitors with nature, an approach termed "landscape immersion." Although the zoo community now focuses on protecting the welfare of their animals and meeting more than their basic needs, some criticize the institution, believing that animals should not be housed in captivity, regardless of the intent of the zoo community (Kreger and Hutchins 2010).

2.1.2 Goals of Zoos

The purpose of many of today's zoological parks goes beyond solely providing entertainment and excitement for visitors through their live animal exhibits. Although zoos entice their visitors with a promise of recreation and leisure, their primary goals now center on education, research, and conservation (Lewis 2010). Many well-run zoos are now institutions where conservation efforts, research, and education of visitors are used to promote and protect threatened wildlife and habitats (Routman et al. 2010). The preservation of biodiversity and subsequent persistence of wildlife populations through these means is the ultimate goal (Conway 2011). A study by Patrick et al. (2010) of the mission statements of 136 AZA accredited zoos reflects this purpose of zoological institutions. Mission statements are meant to guide the actions of zoos so that they can achieve their ultimate goal. Of the mission statements examined, 131 included education and 118 included conservation. The high prevalence of these education and conservation in zoos' mission statements shows the importance of these goals for the zoo community.

2.1.2.1 Conservation

Conservation has become much more important for the zoo community in the last few decades as wild animals and natural habitats continue to decline. Since the 1970s, the population of the Earth's wild animals has decreased by 30% (Conway 2011). With this growing threat of extinction, it is essential to find ways to ensure the survival of wildlife is essential. The World Zoo Conservation Strategy (WZCS), published by the World Zoo Organization (IUDZG) and the Captive Breeding Specialist Group of The World Conservation Union (IUCN) in 1993, set goals for zoos worldwide and showed that the zoo had evolved into an institution centered on the conservation of biodiversity (Tribe and Booth 2003). The WZCS urged zoos to achieve their conservation goals through three initiatives: supporting conservation of endangered species and natural ecosystems, increasing scientific knowledge to benefit conservation, and promoting public awareness of conservation topics (IUDZG/CBSG (IUCN/SSC) 1993). The strategy also suggested ways that zoos could become involved with conservation efforts, both *in situ* (in the wild) and *ex situ* (outside of the natural habitat).

More conservation strategies have been published by the World Association of Zoos and Aquariums since 1993, including *Building a Future for Wildlife: The World Zoo and Aquarium Conservation Strategy* in 2005 (WAZA). In this strategy, WAZA clearly defined conservation as “the securing of long-term populations of species in natural ecosystems and habitats wherever possible” to put emphasis on protecting animals in their natural habitat, not just in captivity (WAZA 2005; Price 2005). Additionally, conservation is meant to preserve naturally occurring biodiversity, or the biological diversity that has occurred due to evolutionary events rather than human intervention (Kreger and Hutchins 2010).

Conservation efforts can be focused both *in situ* and *ex situ*. *In situ* conservation includes protection and restoration of threatened habitats, rescue and protection of endangered species, and reintroduction of species through captive breeding (Tribe and

Booth 2003). Recently, zoos have increased their *in situ* efforts, and many have been successful. For example, the Golden Lion Tamarin Conservation Program at the Smithsonian National Zoological Park was successful at reintroducing captive bred golden lion tamarins (*Leontopithecus rosalia)* into their natural habitats in Brazil (Tribe and Booth 2003). In 1999, the Wildlife Conservation Society (WCS), established at the Bronx Zoo in New York, managed 300 field conservation projects worldwide spanning an array of species. Currently, WCS oversees 500 field projects and manages over 200 million acres of protected land (WCS 2012). Although not all zoos have the resources for *in situ* conservation, many zoos contribute to the efforts financially. Funding for *in situ* projects come from zoo's operational budgets and income as well as visitor donations and admission fees (Tribe and Booth 2003; Conway 2011).

Ex situ conservation includes genetic management and captive breeding, but also relies heavily on the other two goals, research and education (Kreger and Hutchins 2010). Regional and international collaboration between zoos, such as the International Species Inventory System, has allowed for management of the captive populations as a type of genetic "insurance" for declining wild populations (Tribe and Booth 2003). Research studies, ranging from husbandry practices to animal behavior and physiology, help to facilitate programs such as captive breeding to increase the success of conservation. Education is a method of *ex situ* conservation because the general public lacks knowledge about conservation and the other strategies zoos are utilizing to protect biodiversity (Patrick et al. 2010). Conservation education can have a profound effect on the attitudes of zoo visitors towards the animals, the zoo, and the environment (Routman et al. 2010).

2.1.2.2 Research

Research has long been a goal of modern zoos, although the purpose of research has changed as the institution itself has evolved. Zoos provide excellent opportunity for basic research because of the access to a variety of species in a controlled setting. Applied research performed at zoos may be done to meet a variety of needs, whether for the zoo conducting the research or for the zoo community as a whole (Hutchins and Thompson 2008). Recent research topics include behavioral studies, reproductive biology, nutrition, demography, infectious disease or pathology, genetics, veterinary medicine, captive management of wildlife, and environmental enrichment (Hutchins and Thompson 2008; Maple and Bashaw 2010). Zoos provide opportunities to understand and study animals in manners that may not be possible in the wild. The information gained from zoo research can often be applied directly to improving their psychological and physiological well-being (Maple and Bashaw 2010).

Research, whether *in situ* or *ex situ*, also advances the conservation efforts of zoos. Partnerships between zoos and academic institutions allow for a combination of resources and knowledge to accomplish research and advancement that could not have been achieved independently (Kreger and Hutchins 2010; Tribe and Booth 2003). Collaborative efforts with other zoos can also help overcome limitations such as small sample size and limited resources (Kagan and Veasey 2010).

2.1.2.3 Education

In the 20th century, education programs were established at many zoos as the zoo community embraced the philosophy that educating visitors about the animals was the best way to increase their appreciation for wildlife. Zoos learned that live animals had an educational value that other resources could not achieve (Routman et al. 2010; Morgan and Gramann 1989). Until recently, however, zoos focused on simply increasing public knowledge, rather than influencing their values (Ogden and Heimlich 2009). Because zoos are visited by millions of people each year, zoos have a powerful opportunity to both inform the public and shape its attitude towards wildlife (Routman et al. 2010). This is done through education about the animals themselves, their behaviors, their habitats, and their current status in the wild (Patrick et al. 2010). Visitors are informed about the natural history and needs of the animals through the use of signs, interactive exhibits, and live demonstrations such as animal shows or feeding programs (Ballantyne et al. 2007). Some zoos allow direct interaction between the visitors and zoos, such as allowing visitors to feed or touch the animals under the close supervision of a keeper (Routman et al. 2010). Zoos offer programs for students of all ages, teachers, and the general public to encourage learning about the diverse wildlife (Patrick et al 2010).

Educating zoo visitors allows them to form a personal connection with the animals and develop a better understanding of how their own actions impact wildlife and the environment (Routman et al. 2010). It is essential for the public to know what conservation is, the importance of biodiversity for the environment and humans, and the actions that need to be taken to ensure the survival of threatened and endangered species (Patrick et al. 2010). As part of conservation education, zoos also educate the public about their own ability to participate in conservation efforts. Conservation education encourages the public to act in an environmentally responsible manner through involvement with local conservation projects or protection of local wildlife habitats (Zimmerman 2010; Rabb 2004). Zoos need public support and approval to continue their research and conservation efforts. Studies show that education is an effective way of increasing appreciation for animals and their natural habitats, as well as impressing upon visitors the importance of zoos and conveying their role in conservation (Routman et al. 2010).

2.1.3 Laws about Animal Welfare

Laws pertaining to zoo animals exist on international, federal, state, and local levels. The Convention on International Trade of Endangered Species of Wild Flora and Fauna (CITES) is an international statute that regulates the trade of thousands of endangered species. Animals included in CITES are listed in one of three appendices. Species under Appendix I are afforded the most protection, and their trade is illegal. Appendix II species are less strictly regulated, and Appendix III species are afforded even less protection, but are still monitored. With CITES, there is no international regulation: CITES is only as strong as its voluntary member countries. Similarly, the International Air Transport Association regulates the majority of airlines, but membership is voluntary. Participating airlines must meet the standards for shipping live animals to ensure safe transport (Grech 2004).

At the United States federal level, the Animal Welfare Act (AWA, 7 USC, §§ 2131-2159) protects the welfare of individual zoo animals. Under this statute, captive animals are protected by regulations that govern their husbandry and transport. Surprisingly, cold blooded animals are excluded from the AWA definition of animal. Another federal law applicable to zoos is the Endangered Species Act (ESA, 7 U.S.C. § 136, 16 U.S.C. § 1531 et seq), which applies only to species listed as endangered or threatened by the U.S. Fish and Wildlife Service and regulates the import or export of species bought or sold in foreign commerce. This designation is often a factor considered by zoo management in maintaining their collection due to the fact that endangered animals are often flagship species for conservation and education (Grech 2004).

A third federal law applicable to zoos is the Lacey Act of 1900 (18 U.S.C. §§ 41-4817; as amended 16 U.S.C. §§ 3371-3378) which focuses on the prohibition of interstate as well as international trafficking in protected wildlife (Anderson 1995). The Act requires accurate labeling of wildlife shipping and criminalizes most trafficking in fish, wildlife and plants that have been taken or sold in violation of other existing laws. Under the Lacey Act, it is illegal to take, disturb or kill animals from federal sanctuaries, refugees or breeding grounds and prohibits the importation of invasive species into the United States (Balcom 2012). However, there are exemptions under the Lacey Act that permit the taking of animals for the purposes of research, zoo and academic purposes when in possession of a federal permit given the shipping method is deemed humane and healthful for the wildlife (Anderson 1995).

Another minor, though relevant law is the Tariff Act of 1930 (19 U.S.C. § 1527). The Tariff Act prevents the import of animals or animal products obtained illegally in other countries. Importation requires certification that the animal or animal product was taken legally from the country of import and the forfeiture of any animal or product found in violation of this Act (19 U.S.C. § 1527).

2.2 Animal Welfare

In general, welfare is the condition of good health mentally, physically, and emotionally (Kagan and Veasey 2010). According to Hill and Broom (2009), welfare is defined as “the state of an animal as regards its attempts to cope with its environment.”

According to this definition, animal welfare is measurable and can be determined from physiological and behavioral indicators.

The concept of zoo animal welfare was brought to the public's attention by Gillespie (1934) when he published *Is it cruel? A study of the condition of captive and performing animals*, which highlighted the inadequacies in the quality of life of animals in zoos at that time. As public concern for the well-being of captive animal increased, legislation such as the Wild Animals in Captivity Protection Act of 1900/1911 in the United Kingdom was passed. The 1970 Amendments to the U.S. Animal Welfare Act of 1970 were intended to improve animal care throughout the United States, including animals in zoos (Kagan and Veasey 2010).

Many developments in the past century have led to a better understanding of animal welfare and what steps are needed to ensure well-being for captive animals. For example, the importance of the environment to captive animals' well-being was first noted by Robert Means Yerkes in his 1925 book, *Almost Human*, where he chronicled his experiences while living with two chimpanzees in his home. He recognized the importance of an engaging and challenging environment, both physically and socially (Yerkes 1925). Heini Hediger (1950, 1969) further developed this idea, documenting specific principles of management regimes and diet that would further the well-being of animals in captivity. Throughout the rest of the 20^{th} century, many academics continued to publish new insights about animal psychology, eventually contributing to the underlying principles behind modern environmental enrichment. Neuringer (1969) found that animals would often prefer to work for food rather than having it presented to them, suggesting a basic need to search for food. Findings about animal psychology like these provided animal caretakers with ideas they could use to improve the well-being of their charges.

2.2.1 Importance of Animal Welfare

According to Swaisgood and Shepherdson (2006), protecting animal welfare is a now a primary ethic guiding many zoos today. Recently, the zoo community has increasingly recognized the importance of captive animal welfare and has focused more on the psychological well-being of captive animals (Kagan and Veasey 2010). The factors influencing quality of life for a particular animal are idiosyncratic. Although humans often attribute human emotions and desires onto animals, this anthropomorphic approach can be misguided. Zoo caretakers must understand the biology and individual preferences of the animals in their care in order to protect the animals and their quality of life. By focusing on animal welfare and providing animals with more than simply their basic needs, zoos can better achieve their goals of conservation, research, and education. However, deleterious effects of captivity undermine these efforts, causing diminished welfare and hampering zoos' ability to fulfill their purpose.

2.2.1.1 Effects of Captivity on Physiology

According to Cohen et al. (1997), chronic stress occurs "when environment demands tax or exceed the adaptive capacity of an organism, resulting in psychological and biological changes that may place a person or animal at risk for disease." In accordance with this definition, studies have shown that stress and diminished welfare can have a negative impact on overall health (Swaisgood and Shepherdson 2006). If an animal is unable to cope with prolonged stresses, levels of glucocorticoids, a group of hormones used to prepare animals for coping responses, may increase. High hormonal activity can lead to immunosuppression and decreased fecundity, or reproductive function (McPhee and Carlstead 2010). Additional physiological responses experienced by animals in captivity include weight loss, thickening of arteries, atrophy of tissues, elevated blood pressure, and premature death (Kagan and Veasey 2010; McPhee and Carlstead 2010).

Improved animal welfare and treating animals humanely requires protection of the animal's physiological well-being. Keeping each animal safe and healthy is essential, regardless of the zoo goals. Additionally, conservation efforts are also dependent on good physiological welfare. To maintain endangered species' populations and reintroduce captive-bred animals into natural habitats, successful reproduction in captivity is vital (Swaisgood and Shepherdson 2006), and thus limiting stress is essential. Furthermore, death of captive animals affects genetic management efforts as well as the public opinion of zoo conservation efforts. Additionally, healthy animals have more educational benefit for visitors, increasing public appreciation for the animals, their behaviors, and their habitats (McPhee and Carlstead 2010).

2.2.1.2 Effects of Captivity on Behavior

Behavior is considered an animal's "first line of defense" (Mench 1998), allowing the animal to respond to its environment and exert some level of control over it. Complex behaviors have evolved to increase survival and reproductive success in an animal's natural habitat (McPhee and Carlstead 2010); however, captivity may prevent animals from expressing these behaviors, causing frustration and stress (Shepherdson 2010). An animal's welfare is greatly impacted by its ability to express its species-specific behaviors (Kagan and Veasey 2010). Because animals cannot always perform these behaviors in captivity, they often develop other behaviors to cope with stressors in their environment (McPhee and Carlstead 2010). Some captive animals use stereotypical behavior as a coping mechanism to relieve environmentally-induced stress (Swaisgood and Shepherdson 2005). According to Shyne (2006), stereotypic behaviors are repetitive behaviors that lack purpose, yet are consistently displayed by the animal. For example, pacing and head-bobbing are typical stereotypies displayed by captive giant pandas (Liu et al 2006) and captive elephants (Rees 2003). Stereotypic behaviors may also be performed when the behavior an animal would exhibit in its natural habitat, such as foraging, hunting, mating, or escaping from predators and humans, cannot be expressed because of the restrictions and limitations of the captive environment (McPhee and Carlstead 2010; Shyne 2006). For example, animals with large ranging tendencies in the wild are more likely to pace in captivity because their ability to express this natural behavior is inhibited by the limited range of their captive environment (Clubb and Mason 2003).

When zoo animals are inactive and fail to exhibit natural, species-specific behaviors, visitor education becomes less effective (Altman 1998). Captive animals that do not exhibit natural behaviors are not accurate representations of their counterparts in the wild. Also, visitors believe that animals expressing abnormal behaviors such as stereotypies are “unhappy” and conclude that the zoo is not promoting the welfare of captive animals or the preservation of biodiversity (McPhee and Carlstead 2010; Altman 1998). Lack of natural behaviors also has implications for conservation efforts such as reintroduction. If a captive animal has not been able to express natural behaviors vital to its survival in the wild, the animal will be at a disadvantage once reintroduced and may die prematurely (McPhee and Carlstead 2010).

2.2.2 Assessing State of an Animal's Welfare

As the definition of welfare provided by Hill and Broom (2009) implies, welfare can be measured using physiological and behavioral indicators. Physiological indicators may be as basic as injuries the animal has sustained, weight loss, or other abnormalities of essential physiological functions. Measuring glucocorticoids levels in blood, urine, or feces is a common method of assessing stress in animals due to the elevation of hormone levels that occurs in response to stress (Hodges et al. 2010; McPhee and Carlstead 2010). An animal's welfare can also be judged by comparing its behavior and the amount of time spent performing various behaviors (its activity budget) with the natural behaviors expressed by its wild counterparts. The presence of abnormal behaviors, such as stereotypic behaviors, or the absence of survival and reproductive behaviors also indicates diminished welfare (Kagan and Veasey 2010). However, according to Swaisgood and Shepherdson (2005), if the captive environment is deficient, animals displaying stereotypic behavior may have better welfare than animals in the same environment that do not display stereotypic behaviors, because the stereotypic behaviors allow the animals to cope with the aversive conditions.

2.2.3 Methods of Improving Animal Welfare

Captive animal welfare can be improved by providing the animals with environments that are designed to meet their physiological and psychological needs. This can be done in part through the design of the enclosure itself. Although the animals live in captivity, their environment should not be unnecessarily confining. The animals should not be exposed to stressors such as proximity to predators, extreme space restrictions, or excessive sensory stimulation (McPhee and Carlstead 2010), and the potential for pain and fear should be minimized (Fraser 2009). Also, the animals should be able to exercise some level of control over their environments and be able to express their natural behaviors (Kagan and Veasey 2010). Mimicking the natural habitat in the enclosures and implementing environmental enrichment can increase the expression of natural behaviors (Shepherdson 2010). Because stereotypic tendencies are seen as an indication of a potential decline in an animal's well-being, environmental enrichment has been used to reduce these behaviors and decrease perceived stress in captive animals (Swaisgood and Shepherdson 2005).

2.3 Accreditation

The emerging science of animal welfare has provided a foundation for zoo management through standardized practices and protocols (Maple 2007). To gain accreditation and a permit to possess exotic animals, a zoo must meet certain animal welfare and care standards. There are several forms of accreditation including that issued by the Association of Zoos and Aquariums (AZA) and the Zoological Association of America (ZAA). Each of these organizations has developed accreditation requirements and standards a zoo must meet or exceed to maintain accreditation.

2.3.1 AZA Accreditation

The Association of Zoos and Aquariums is the primary accrediting body for zoos and aquaria in the United States. AZA accreditation is highly respected due to its rigorous requirements (AZA 2012). Of the top ten zoos in the USA Travel Guide, nine have obtained AZA accreditation (USA Travel Guide 2009). AZA was founded in 1924, while the other zoo accrediting organizations are much more recently formed. AZA is a nonprofit organization that focuses on advancing care standards for captive species through the areas of conservation, education, and science. At the last Accreditation Commission hearing in September 2011, the current number of AZA-accredited zoos and aquaria totaled 225 (AZA 2011), and each must undergo an inspection once every five years to remain accredited. AZA accredited institutions must comply with all regional, state and national laws, although the AZA accreditation process ensures that these facilities often meet higher standards of animal care than are required by law. According to accreditation standards, the animals must be displayed in naturalistic settings while providing an educational experience for visitors. This setting must be coupled with an "appropriate enriching environment" for the animals, which includes maintaining them in species appropriate social groupings. Zoo collections must be managed in a way that sustains long-term genetic viability through a combination of *ex-situ* breeding and *ex-situ/in-situ* conservation and research (AZA 2011).

Animal care manuals must be accessible at every AZA accredited institution for each species in their collection. These manuals contain requirements, guidelines and suggestions for the proper housing and care of each species. Daily logs of activities, food intake and other specifics recorded by animal care staff are required for each identifiable animal and are used to enhance husbandry and breeding conditions (AZA 2011).

Other factors considered for accreditation are housing, socialization, and operant conditioning. Operant conditioning is used to train animals to respond to human commands, with the ultimate goal of enabling basic husbandry routines and medical procedures to be accomplished without undue stress. It can also be used to encourage higher activity levels and natural behaviors in species that become lethargic in captivity. For example, large cats have been trained to chase catapulted meatballs. Operant conditioning can also encourage animals to present body parts for examination or blood withdrawal, thereby decreasing the stress involved in medical examinations. Ideally, operant conditioning is used to improve the interactivity and novelty of the zoo environment for zoo animals (Maple 2007).

Because one of the main goals of a zoo or aquarium is the education of the public, there are special conditions mandated by AZA regarding the animals used in education programs. Adequate measures must be taken to ensure the animal is not exposed to infectious agents. The housing conditions of animals used in zoo education programs are often different than those of exhibit animals, but their physical and psychological needs must still be met (AZA 2011). A large section of the AZA Accreditation Standards of 2011 pertains to the institution having a "clear process for identifying, communicating, and addressing animal welfare concerns . . . in a timely manner" (AZA 2011; AZA 2012).

2.3.1.1 AZA Enrichment Standards

According to the Behavior Advisory Group of the AZA, environmental enrichment is considered a dynamic process of "improving or enhancing zoo animal environments and care within the context of their inhabitant's behavioral biology and natural history" with the purpose of eliciting species appropriate behaviors and benefitting an animal's welfare (Shepherdson 2010). The AZA has set minimum guidelines for accredited zoos to follow to ensure animal welfare and uphold the goals of the zoo community. The institution must have a formal enrichment program that encourages species appropriate behaviors. The enrichment plan must be based on the most recent understanding of an animal's biological needs and should be updated regularly to reflect advances in knowledge that may impact the success of enrichment. The plan should also include several elements, such as a planning and approval process, implementation, evaluation and subsequent program refinement. A specific staff member or committee must be assigned to oversee the enrichment program through implementation, training, and coordination between departments (AZA 2011).

2.3.2 ZAA Accreditation

The Zoological Association of America was formed in 2005 and provides an accreditation process similar to that of AZA. A total of 43 institutions are currently accredited by the ZAA (ZAA 2008). The objectives of ZAA accreditation are to maintain professional standards of husbandry practices and accurate animal/medical records, enhance the survival of the species, and maintain a "quality existence" for captive animals while ensuring the safety of both staff and visitors. For an institution to apply for ZAA accreditation, they must first be members of ZAA and pay the required dues (ZAA 2008). Similar to AZA accreditation, ZAA accredited zoos are subject to inspections to ensure adequate facilities, record keeping, animal nutrition and licensing.

The ZAA sets specific standards for each species by designating them as Class I, II or III based on enclosure and care requirements. Class I includes some primates, felids, and other large mammals, and the facilities requirements are very specific. ZAA members are encouraged to notify the organization of new, innovative programs they believe will help establish better care practices among ZAA institutions. Because of this policy, the standards listed for ZAA accreditation are dynamic. Inspections occur every five years and are conducted by a pair of ZAA representatives (ZAA 2008). Based on the differences in rigor between ZAA and AZA requirements (AZA being the more rigorous), it is conceivable that some zoos may choose ZAA accreditation over AZA.

2.4 Environmental Enrichment

Although zoos have a long history, it is only recently that the term "environmental enrichment" has entered the zoological community's vernacular. There are several different definitions of the term and different viewpoints on exactly what the goal of enrichment should be.

Newberry (1995) claimed that environmental enrichment should be a descriptor not of changes made to the environment, but of the outcome of those changes. According to Newberry, rather than describing the act of enriching the environment, the term indicates that the environment is enriching the animals. In considering various approaches to enrichment, Newberry downplays the usefulness of enrichment promoting natural behaviors, because the species-typical behaviors that are beneficial for an animal in the wild may not prove beneficial in the captive environment. She also states that while reducing negative emotional states is a worthy goal, the difficulty in measuring emotional states makes it concretely impossible to judge the effectiveness of enrichment this way. The definition provided by Newberry (1995) requires that the modifications to the animal's environment have a beneficial impact on the animal's well-being, demonstrated through improved health, reproductive success, and fitness. Boissy et al. (2007) echoed Newberry's sentiment about concrete improvement in animal welfare, saying that enrichment must be differentiated from simple "housing supplementation," and that "the term 'enrichment' should be reserved for environments that are truly enriched beyond basic needs." However, Boissy et al. (2007) focused on the presence of positive emotions as the best measure of success, assessing the presence of these positive emotions through hormonal measures and the expression of behaviors such as play, affiliative behavior, and grooming.

Mellen and MacPhee (2001) presented a goal-based definition of enrichment, rather than the results-based definitions favored by Newberry (1995) and Boissy et al. (2007). Their five listed goals are:

(1) Enhancing the psychological and physiological well-being of animals
(2) Having animals which successfully reproduce and exhibit adequate parental care
(3) Identifying and reducing potential sources of chronic stress
(4) Reducing or eliminating aberrant behaviors
(5) Aiding the re-introduction of captive-born animals to their natural habitats

They further state that the exact nature and measurement of these goals must be tailored to individual establishments and animals, creating a generally holistic approach to enrichment.

For the purposes of this study, we follow the definition of Van Metter et al. (2008), who describe enrichment as "the dynamic process that structures and changes an animal's environment in a way that provides for behavioral choices and elicits species' natural behaviors and abilities from the animal."

2.4.1 History of Enrichment in Zoos

Despite the increased focus on animal welfare and psychology throughout the 20th century, environmental enrichment itself remained largely the domain of individual animal keepers until the 1980s. Enrichment techniques were typically communicated informally from keeper to keeper, and peer-reviewed studies were relatively rare (Swaisgood and Shepherdson 2005). Several researchers, including Hal Markowitz, began using systematic data collection to determine how captive animals best thrive in captive environments, and publications such as *Animal Keepers' Forum* and *Ratel* provided a medium for zookeepers and academics to share environmental enrichment ideas (Mellen and MacPhee 2001). In the 1990s, the first conference on environmental enrichment was held, and the first book specifically on environmental enrichment, *Second Nature: Environmental Enrichment for Captive Animals*, was published (Shepherdson et al. 1998).

While the results of environmental enrichment are now studied with more controlled, scientific methods (Shepherdson et al. 1998), it has still been difficult for the zoological community to find evidence for which enrichment strategies are most effective. Swaisgood and Shepherdson (2005) attribute this difficulty to two things. First, any use of environmental enrichment is tailored to the specific needs of the individual animal or species, making it difficult for researchers to make generalizations about the most effective enrichment methods. Additionally, although recent studies of environmental enrichment tend to be rigorously designed, they are still relatively uncommon.

2.4.2 Types of Enrichment

There are many different types of enrichment, but all are intended to improve animal welfare and stimulate various natural behaviors. Hoy et al. (2010) recognized eight categories of enrichment: feeding, tactile, structural, auditory, olfactory, visual, social, and human-animal interactions. Visual, olfactory, and auditory enrichment are collectively referred to as sensory enrichment (Hoy et al. 2010). Examples of sensory enrichment include playing recorded animal sounds or music, distributing scents within the exhibit, or placing mirrors within the exhibit to allow animals to view their reflections. Scent may include artificial scents such as perfumes and spices, or natural products such as urine and feces from other species (Clark and King 2008). Feeding enrichment is defined as a manipulation of the food or method of introducing the food to the animal that allows an animal to express more natural feeding behaviors. This can be accomplished with task-oriented puzzle feeders to encourage the animals to work for their food or by hiding and scattering the food around the enclosure. In addition, whole animal carcasses or animal blood, in the form of a bloodsicle (Figure 2.1), may be given to larger carnivores.

Figure 2.1 A tiger interacts with a bloodsicle (a frozen block of blood). Photo from Meghan Murphy, National Zoological Park.

Another form of enrichment closely related to feeding is tactile, also referred to as manipulative enrichment. Manipulative enrichment allows the animal to explore, play, and express other species-specific behaviors with novel objects including bags, boxes, barrels, and other toys. For example, cardboard piñatas are given to the lions to promote the predatory behaviors they would express in their natural habitat (Figure 2.2). Often, manipulative enrichments are combined with sensory or feeding enrichments, such as stuffing paper bags with scents or fresh meat.

Figure 2.2 A lion interacts with a cardboard piñata, an example of manipulative enrichment. Photo from Houston Zoo.

To mimic the animals' natural habitats, zoos include structural enrichment. Structural enrichment includes adding natural substrates such as ground coverings, water features, trees for climbing (Figure 2.3) and caves for hiding. Finally, interactions between animals and between humans and the animals are considered forms of behavioral enrichment. For example, positive reinforcement training and operant conditioning allows interaction between keepers and the animals in their care (Hoy et al. 2010).

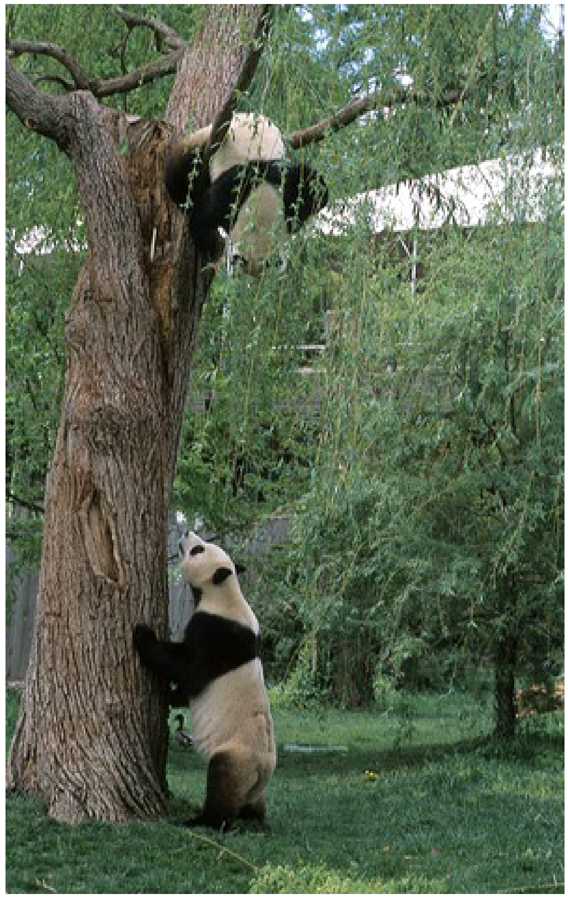

Figure 2.3 Natural elements such as trees in the exhibit allow pandas to express natural behaviors. Photo from National Zoological Park.

Hoy et al. (2010) conducted an international survey of individuals involved in animal care, husbandry, and research to determine the current enrichment practices most commonly used for mammals. Survey respondents considered feeding enrichment to be the most important, followed by tactile, structural, and olfactory techniques. Olfactory enrichment was the most important of the three types of sensory enrichment. The most frequently used enrichments were feeding, human-animal interactions, and tactile. Although structural enrichment was considered important, the difficulty of making changes to the enclosure made the implementation of structural enrichment less common. Hoy et al. (2010) also surveyed individuals regarding factors that limited the frequency and amount of enrichment used. The most limiting factor mentioned by keepers and zoo personnel was the amount of time available. Most spent an hour or less per day on enrichment practices and focused most of their efforts on primates and carnivores. Additionally, the survey respondents indicated that they lacked the time to evaluate the effectiveness of enrichment, which might deter keepers from trying new types of enrichment. Hoy et al. (2010) concluded that the zoo community felt that increased quantity and variety of enrichment was needed, but animals were not provided with enrichment as frequently as the zoo staff would have liked due to limiting factors such as time and manpower. These factors also limited the evaluation of enrichment used.

Existing research on the behavior and welfare of captive animals has led to more enlightened zoo husbandry practices. Zoos have successfully manipulated many factors, such as social density, enclosure design, and the animal's degree of autonomy, to provide more stimulating environments for animals (Maple 2007). However, despite these advances, many zoos, especially those lacking the necessary monetary and human resources, have difficulty establishing successful enrichment programs (Fuchs and Ray 2008). Manpower and time are two factors that contribute to this difficulty (Hoy et al. 2010). Thus, enrichment must be easily implemented and demonstrably effective to be feasible.

2.4.3 Measuring the Success of Enrichment

The effectiveness and success of enrichment programs can be measured in a variety of different ways. The first is the reduction of stereotypic or abnormal behaviors usually linked with poor animal welfare (Shepherdson 2010). There is a growing body of literature showing that enrichment is an effective method of significantly reducing stereotypic behavior (reviewed by Swaisgood and Shepherdson 2005). Because environmental enrichment has been shown to help reduce stereotypic behavior in captive animals, a decrease in these behaviors following implementation of an enrichment program would be an indicator of its success (Swaisgood and Shepherdson 2006).

Decreasing stereotypic behaviors alone does not indicate improved welfare, however. Captive animals tend to exhibit less diverse behavior than their wild counterparts, so an increase in the captive animal's behavioral diversity can also be used to evaluate enrichment efforts. The more varied these species-specific behaviors are, the more effective the enrichment program. An activity budget, determined by the amount of time spent performing a variety of defined behaviors, similar to that of an animal's wild counterpart would indicate improved welfare and therefore successful enrichment (Kagan and Veasey 2010). Additionally, the amount of enclosure space the animal uses can be used to judge the effectiveness of the structural enrichment and enclosure design (Mallapur et al. 2002). Finally, reproductive success and a reduction in physiological indicators of stress (i.e., stress hormones such as cortisol) may indicate a successful enrichment program (Moreira et al 2007).

2.4.4 Effects of Enrichment on Behavior and Physiology

The effects of the different types of enrichment (feeding, sensory, tactile, structural, and human-animal interactions) on animals' psychological and physiological welfare have been studied in various species.

Feeding enrichment has been shown to have an impact on stereotypic behavior of captive animals. Bashaw et al. (2003) found positive effects of feeding enrichment in African lions and Sumatran tigers that were given either live fish or horse leg bones twice per week. Both variety and frequency of feeding behaviors increased, while stereotypic pacing decreased by two fold. The effects of this enrichment lasted for up to two days, indicating the ability of feeding enrichment to alter the animals underlying activity patterns.

However, McPhee (2002) found a more limited effect of feeding enrichment on stereotypic behavior. McPhee researched the effects of intact carcasses on nine felids: three African leopards (*Panthera pardus pardus)*, two African lions, and four snow leopards (*Panthera uncia*). During the study, the felids received a carcass every two weeks for a total of seven carcasses over a period of fourteen weeks. Off-exhibit stereotypic behavior decreased compared to baseline, while on-exhibit behaviors were largely unaffected. A similar experiment measured the effects of various types of enrichment on six species of felids: cheetahs (*Acinoynx jubatus*), cougars, jaguars (*Panthera onca*), lions, ocelots (*Leopardus pardalis*), and tigers. Skibiel et al. (2007) recorded the effects of bones, frozen fish, and spices on activity levels and stereotypic pacing and found that each form of enrichment stimulated activity levels. Stereotypic behaviors decreased only with the addition of spices and frozen fish.

As demonstrated by Skibiel et al. (2007), sensory enrichments also impact animal behaviors. Another study using olfactory enrichment by Wells and Egli (2004) found that olfactory enrichment had a positive effect on the behavior of captive black footed cats (*Felis nigripes)*. The cats were subject to four olfactory conditions: no odor (control), nutmeg, catnip, and odor of prey. They concluded that all of the experimental odors increased the amount of time the cats spent active and decreased sedentary behaviors. Resende et al. (2011) tested the effects of two odors, catnip and cinnamon, on eight oncilla cats (*Leopardus tigrinus)*. Duration of stereotypical pacing was recorded before, during, and after the experiment. Catnip had no effect on stereotypical pacing, but cinnamon reduced pacing during and after the experiment.

Wells et al. (2006) analyzed the effects of auditory stimulation, another form of sensory enrichment, on the behavior and welfare of captive Western lowland gorillas (*Gorilla gorilla gorilla*). Gorillas were exposed to three auditory conditions: no auditory stimulation (control), an ecological sound (such as that found in the natural habitat), and a non-ecological sound (such as classical music). With auditory stimulation, the expression of relaxation behaviors, such as resting and sitting, and socializing, increased, while stress-induced behaviors decreased. However, these trends were not statistically significant when compared to behaviors performed in the absence of auditory stimulation. Wells et al. (2006) hypothesized that the presence of new auditory stimulation "masked" other auditory stressors, such as noise from visitors.

The complexity of enclosures and presence of enrichment has been shown to have an impact on physiological functions in addition to behaviors. Moreira et al. (2007) found that changes in the size of environmental enclosures caused the reproductive cycle in female tigrinas (also known as oncilla) and margays (*Leopardus wiedii*) to change drastically. The animals were first placed in a large environment with enrichment, such as branches, plants, and nest boxes. These items allowed for expression of natural behaviors such as exploratory and territorial behaviors, as well as stress coping mechanisms. The animals were then transferred into smaller enclosures without enrichment for several months. During the final phase of the study, the animals were kept in the small enclosures, but similar enrichment to that in the larger enclosures was added. Throughout the study, fecal corticoid and estradiol levels were measured. Elevated corticoid concentrations were used as an indicator of stress, while decreased estradiol levels were due to reduced ovarian activity. Both species had a dramatic increase in corticoid concentrations when moved to the small, empty enclosures. Additionally, both species experienced a decrease in reproductive activity. The study showed that normal physiological functions, such as reproductive activity, can be greatly disrupted by changes in the environment and a lack of complexity.

Environmental enrichment has been shown to affect neuronal plasticity in laboratory animals. Van Praag et al. (2000) studied the effects of environmental enrichment on neuronal plasticity using two groups of adult rats. One group received enrichment involving wheels for running and tubes for hiding while the other group received no enriched treatment. Both groups received human interaction. The brains were then analyzed to compare brain structures. Researchers concluded that environmental enrichment positively affected the neuroanatomy of the rats. Effects on neuroanatomy included increased brain weight and dendritic branching, and enhancement of cell proliferation and neurogenesis. This may have implications for the benefits of enrichment when used with captive animals in zoos.

2.5 Felids

Members of the *Felidae* family in the mammalian order Carnivora can be found all over the world excluding polar regions, oceanic islands, and the land masses of Australia, New Zealand, Madagascar, and Japan (Feldhamer et al. 2007). Felids (commonly referred to as cats) can range in head and body length from 337 to 2800 mm with a tail anywhere from 51 to 1100 mm long, and their body masses range from 1.5 to over 300 kg (Nowak and Paradiso 1983). Though species vary in size, felids are very similar in body structure and behavior.

Felids have rounded heads and flexible, muscular bodies. Their pelage varies in color, but often has stripes or spots to enable camouflage while hunting (Feldhamer et al. 2007). Felids have eyes with vertically contracting pupils and have keen senses of sight and hearing. Their tongues are covered in papillae, which help retain food in the mouth as well as keep their coats clean (Nowak and Paradiso 1983). They are carnivorous with sharp teeth used to kill their prey. At the base of their tongue, felids have flexible cartilage instead of a hyoid bone. This allows large felids to roar and smaller felids to purr (Feldhamer et al. 2007). A felid's forefoot has five digits and its hindfoot has four, all with retractile claws (Nowak and Paradiso 1983). Their feet are padded and they move very quietly.

Felids stalk or ambush their prey, which can be any mammal or bird. Some species have been known to hunt fish or reptiles. Most species maintain a solitary home range but each individual's home range typically overlaps with the home ranges of other individuals, giving them many opportunities for interaction through olfaction. Typically, kittens leave their mother as soon as they can hunt on their own. Additionally, most, but not all, species are nocturnal. However, there are exceptions (e.g., lions live in prides and cheetahs are diurnal) (Nowak and Paradiso 1983). Many species of felids have become endangered, and several are threatened by issues such as habitat loss and poaching. Some are hunted for their fur, while others are viewed as a threat to humans and forced out of their habitats (Nowak and Paradiso 1983).

2.5.1 Lions

The African lion is the second largest member of the *Felidae*. On average, male lions weigh between 150 and 250 kg, while females weigh between 120 and 182 kg. A fully mature male lion has a head and body length of approximately 1700 to 2500 mm long, with a 900 to 1050 mm tail. Females are about 1400 to 1750 mm in head and body length, and have a tail that is 700 to 1000 mm long (Nowak and Paradiso 1983). Males and females both have compact, muscular bodies built for hunting large prey, rather than for speed; however, they can reach speeds above 50 km/h over short distances. Both sexes have a solid yellow-gold coat, but only males have a mane (Figure 2.4) and tufted tail (Nowell and Jackson 1996). The color of a lion's mane may vary from gold to reddish-brown or black depending on the lion's age (Nowak and Paradiso 1983). Hormones, such as testosterone, influence the color and growth of the mane (Schaller 1972). Lions typically live longer in captivity than in the wild, with an average lifespan of 20 years in captivity compared to 15 years in the wild (Haas et al. 2005). Females have a slightly longer lifespan in the wild than males.

Figure 2.4 Male African lion. Photo from Chris Johns, National Geographic.

2.5.1.1 Habitat

Lions are capable of living in a variety of habitat types, from very arid semi-desert environments to dense woodlands (Schaller 1972). Other than a small population of lions living in the Gir Forest in India, wild lions are currently found solely in Africa (Nowell and Jackson 1996). Their habitat extends through the savanna grasslands of sub-Saharan Africa, with the largest populations concentrated in southern and eastern Africa in such areas as the Serengeti ecosystem (Figure 2.5, Bauer and Van Der Merwe 2004). Smaller, fragmented populations live in western and central Africa. These regions are characterized by open plains, with thick bush and tall grass, which provide coverage during hunting and denning. Historically, lions inhabited regions all over Africa, as well as several other continents. The lion disappeared from Europe in the 1st century AD, but inhabited areas in North Africa, the Middle East and Asia until the 1800s (Bauer and Van Der Merwe 2004). Due to habitat loss, human threats, and disease, lions are now rarely found outside of protected areas (Nowell and Jackson 1996). According to an inventory performed in 2004, between 16,500 and 30,000 free ranging lions currently live in Africa (Bauer and Van Der Merwe 2004). Because of the rapid population decline in the last few decades, lions have been listed as "vulnerable" on the IUCN Red List of Threatened Animals (Bauer and Van Der Merwe 2004).

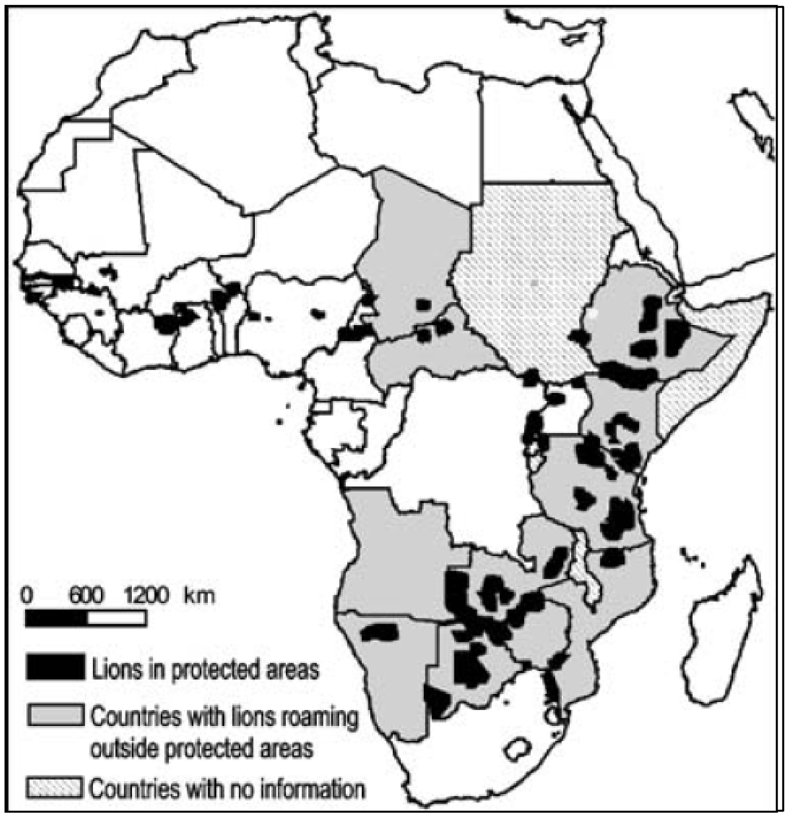

Figure 2.5 Map of existing lion populations in Africa as of 2004 (Bauer and Van Der Merwe 2004).

2.5.1.2 Social Behavior

Unlike other members of the *Felidae*, lions are extremely social mammals. They live in prides, which are comprised of related female lions (Wilson 2000). Females usually remain in the same pride for their entire life. All cubs born into the pride are raised by all of the lions forming the group. Because of this, lions are more successful than other felids at raising their young (Wilson 2000). Once males reach maturity at around two years of age, they leave their natal pride and become nomadic. A male may take over another pride, but control over it typically lasts only two to three years (Nowak and Paradiso 1983). When not in a pride, males are very social with others, and they form alliances with other males from their natal pride (Nowell and Jackson 1996). Within the social group, males and females have distinct roles. Males are responsible for protection from outside males, while females are the primary hunters. While males are nomadic, the territory of the pride is passed down through generations (Wilson 2000). The territorial range may extend from 20 to 400 square kilometers (Nowak and Paradiso 1983). The size of their range is largely dependent on the amount of prey available, and they may travel long distances in order to meet their dietary requirements (Gittleman and Harvey 1982; Hayward and Kerley 2005).

2.5.1.3 Hunting Preferences and Behaviors

Because of their size and power, lions are well suited for hunting medium to large prey. The preferred prey of lions include buffalo *(Syncerus caffer)*, wildebeest (*Connochaetes taurinus*), zebra (*Equus burchelli)*, gemsbok (*Oryx gazelle*) and other species of antelope, and giraffe (*Giraffa camelopardalis)*, although they may hunt species as small as warthog (*Phacochoerus africanus*) and as large as rhinoceroses (*Ceratotherium simum)* and elephants (*Loxodonta africana)* (Hayward and Kerley 2005). Hunting is done mostly by the females within a pride, and they often hunt in groups (Figure 2.6, Wilson 2000). According to Hayward and Kerley (2005), most species of lions prefer their prey to weigh around 350 kg. Group hunting strategies allow lions to select prey much larger than this preferred weight range (Nowak and Paradiso 1983), which enables them to maximize their nutrient intake. Lions typically hunt at night or during the cooler periods of the day. They are visual hunters, and they utilize environmental features of their habitats, such as long grass, to enable them to stalk and approach their prey before attacking (Sunquist and Sunquist 1997; Hayward and Kerley 2005). In captivity, lions are often fed beef and bones, along with other meats.

Figure 2.6 Female lions using a group hunting strategy to surround prey. Photo from Animal Planet.

When not hunting, lions are relatively inactive, though other active behaviors include cub rearing and defense of their territory (Heinsohn and Packer 1995). They have very irregular activity patterns, but their behavior is typically nocturnal or crepuscular, with most activity occurring during late evening or early morning (Gittleman and Harvey 1982; Nowak and Paradiso 1983). Their behavior is highly dependent on their habitat and the season, but they spend much of their time resting. In the wild, lions spend around 20 hours inactive each day, while captive lions sleep 10 to 15 hours per day (Rees 2011).

2.5.2 Tigers

Tigers are the largest members of the *Felidae*, usually ranging from 1400 to 2800 mm in head and body length with tails ranging from 600 to 950 mm. Males can weigh up to 306 kg, and females can weigh up to 167 kg, however there is variation between subspecies in size. Tigers have an anatomy similar to those of other large cats, with muscular legs and a relatively large head. Both males and females coats' are reddish orange to reddish ochre with white or cream undersides. They have black, brown, or gray stripes covering their heads, bodies, tails, and limbs (Figure 2.7, Nowak and Paradiso 1983). They reach maturity at four to five years and their lifespan in captivity is typically 20 to 26 years, a figure consistent with data from the wild (Mazak 1981).

Figure 2.7 Siberian Tiger. Photo from Joel Sartore, National Geographic.

2.5.2.1 Habitat

Tigers generally need only water, plant cover and abundant prey in order to flourish, which allowed them to inhabit many different environments. Historically, tigers were found in south and Southeast Asia, the Indonesian islands, eastern China, Siberia and portions of western Asia surrounding the Caucasus and Caspian Sea. During the 20th century, however, habitat loss and hunting by humans diminished the population numbers and limited them to pockets in south, Southeast and East Asia, with a small population on the island of Sumatra (Figure 2.8, Mazak 1981). Estimates of the tiger population have dropped precipitously from 100,000 at the end of the 19th century to a more current estimate of no more than 7,700 (Nowell and Jackson 1996).

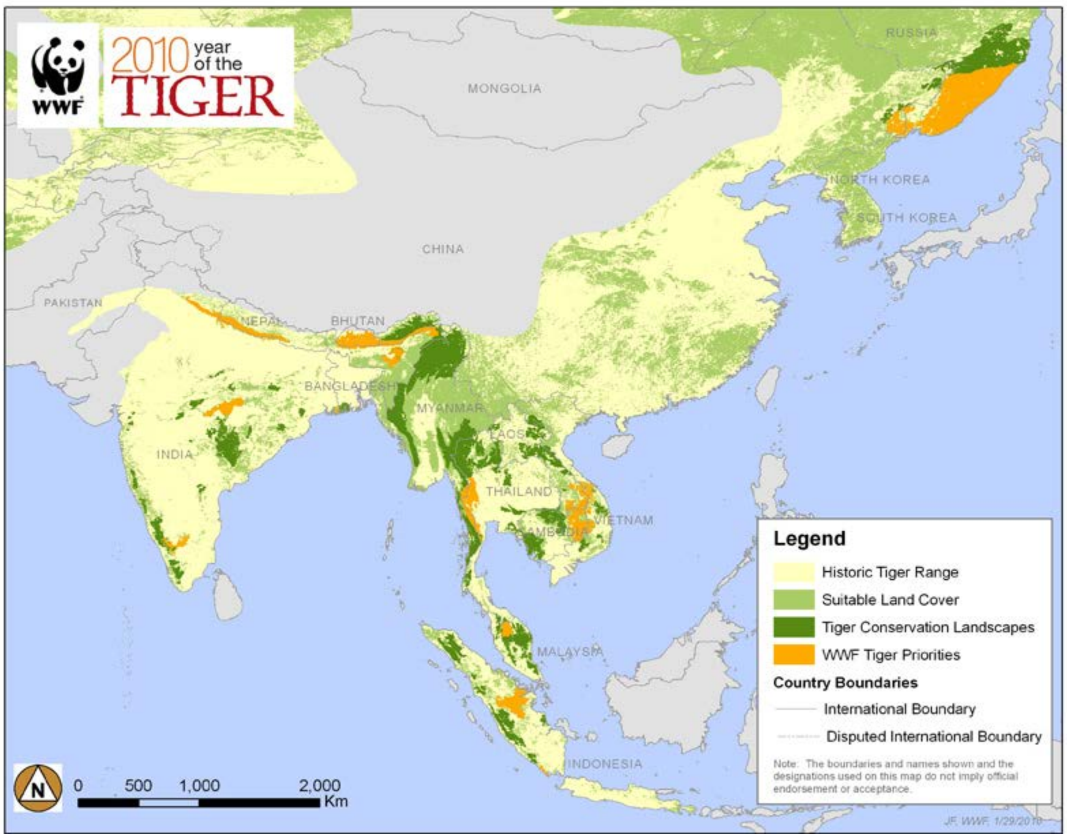

Figure 2.8 Map of current and historic tiger ranges. Map from the World Wildlife Foundation, 2010.

2.5.2.2 Social Behavior

Tigers are usually solitary animals apart from mating and when cubs are dependent on their mother. The mating season takes place between the end of November and the first two weeks of April. Cubs remain with their mothers for approximately two years (Nowak and Paradiso 1983). Despite the lack of regular socialization, tigers are usually aware of the movements of tigers in their surrounding area due to complex territorial marking behavior, usually via urine or claw markings. Unlike most carnivores, tigers tend to retain priority rights over their meat supply, even when faced with a larger and stronger animal, reflecting their solitary nature (Mazak 1981). A single tiger can have a ranging distance of 50 to 4000 km^2 depending on the subspecies and location (Nowak and Paradiso 1983).

2.5.2.3 Hunting Preferences and Behaviors

Tigers generally hunt larger animals such as wild boar (*Sus scrofa)*, Siberian moose (*Alces alces)*, Indian buffalo (*Bubalus bubalis),* and black bears (*Ursus thibetanus)*. Hunting is generally done nocturnally, and while tigers are among the most successful hunters in *Felidae*, they generally make 10 to 20 attempts per kill (Mazak 1981). After the kill, a tiger usually drags its prey to an area with cover (Figure 2.9, Nowak and Paradiso 1983). While hunting, tigers rely mostly on their auditory and visual senses rather than their olfactory capabilities as most other carnivorous hunters do (Mazak 1981).

Figure 2.9 A Bengal tiger carries a chital fawn. Photo from Michael Nichols, National Geographic.

2.5.3 Cougars

Cougars, also known as mountain lions or pumas, are the largest of the small cats. On average, the males weigh 67 to 103 kg and range in head and body length from about 1050 to 1959 mm with a tail length up to 784 mm, while females weigh 36 to 60 kg and have a head and body length of 966 to 1517 mm with a tail length of up to 815 mm (Nowak and Paradiso 1983). Cougars have muscular hind legs, which are proportionally the longest of any felids (Figure 2.10, Nowell and Jackson 1996). These legs, combined with a flexible spine and sharp claws, give cougars increased jumping power and an ability to quickly change direction (Nowak and Paradiso 1983). Although cougars have spotted coats at birth, both genders develop a solid coat, which can be a grey, tawny, or reddish, as they grow. A cougar reaches maturity at two years, with a lifespan of ten years in the wild (Nowell and Jackson 1996) and up to nineteen years in captivity (Nowak and Paradiso 1983).

Figure 2.10 A cougar surveying its surroundings. Photo from Jim and Jamie Dutcher, National Geographic.

2.5.3.1 Habitat

Although studies have shown cougars prefer areas with dense vegetation, they are able to survive in a variety of habitats including deserts, rainforests, coniferous forests, and swamps. Because of this, cougars are among the largest ranging cats. In the past, cougars populated the entirety of North America, but due to hunting practices and threats to their habitat, they are limited to western Canada, the western United States and Florida, Central America, and South America (Figure 2.11, Nowell and Jackson 1996). IUCN has classified the cougar as endangered, with only 16,000 extant cougars (Nowak and Paradiso 1983).

Mountain Lion Range

Figure 2.11 Map of current cougar ranges. Photo from National Geographic Society.

2.5.3.2 Social Behavior

Excluding times where they are breeding or mothers are raising cubs, cougars are solitary. Because a cougar's home range can span up to 90 km^2, two cats may occupy the same territory, but they will consciously avoid each other. There is no specific breeding season, but it is common for females to give birth every two years. Kittens leave their mothers a few months after they become able to make their own kills, but they may stay with littermates for two or three months after leaving (Nowak and Paradiso 1983).

2.5.3.3 Hunting Preferences and Behaviors

A cougar's diet is dependent on its habitat, but they are carnivorous mammals. They are known to eat larger animals such as elk (*Cervus canadensis)* and other species of deer as well as smaller animals like different species of rabbits (such as *Sylvilagus nuttallii)* and beavers (*Castor canadensis)* (Nowak and Paradiso 1983). Cougars are nocturnal cats and their activity levels peak during the dusk and dawn hours (Nowell and Jackson 1996). A cougar hunts by stalking and quickly leaping or pouncing on top of its prey. It will cover its kill and, depending on size, return to the carcass in the following days (Nowak and Paradiso 1983).

2.5.4 Felids in Captivity

Although each species of felid has its own unique characteristics, there are several traits that they share. Therefore, captive felid enclosures tend to share similar design features. Shoemaker et al. (1997) established guidelines for all AZA accredited zoos for keeping large felids in captivity.

In these guidelines, most species have the same specifications for temperature, lighting, ventilation and humidity, water, sanitation, food, and veterinary care. Animals kept in outdoor enclosures should always have access to shade, while those housed indoors should not experience temperatures above 85 degrees Fahrenheit. It is also recommended that indoor enclosures use fluorescent lighting and maintain a humidity of 30 to 70%. All enclosures should be cleaned daily, and drinking water should always be accessible. Felids should be fed almost daily (fasting one or two days a week to avoid obesity is acceptable) with a diet of beef or horse products or a whole animal carcass. It is recommended that bones be given one to two times a week to promote good oral hygiene. A veterinarian should always be available and each animal should receive periodic examinations during the year. Since most felids are solitary, they should be kept singly or in pairs. Lions may be kept in larger groups, but multiple males should not be kept together in any species (Shoemaker et al. 1997).

Some features of the enclosures depend on the species. Larger cats like lions and tigers should be kept in cages at least 20 feet wide and 15 feet deep or in large outdoor enclosures with moats separating the animals from the public. They should be provided with jump walls of at least 16 feet (Shoemaker et al. 1997). Smaller felids, such as cougars, should be housed in enclosures that are at least 200 square feet with secure top covers. Because many of the smaller felid species live in arboreal or rocky habitats, their enclosures should have ledges or perches for sleeping. Cheetahs are a unique species that benefit from very large, spacious outdoor enclosures, but can also be kept in cages of at least 200 square feet (Shoemaker et al. 1997).

Many studies have shown the effects of captivity on the welfare of felids (e.g., Bashaw et al. 2007; Clubb and Mason 2007; Terio et al. 2004). Felids cope with captivity particularly poorly. In a multi-species study of infant mortality rates of animals in captivity, three of the four most at-risk species were felids (Clubb and Mason 2007). Most large felids, with the exception of lions, are solitary carnivores, making the introduction of mates a potentially dangerous process that often leads to fighting, injury, or death (Shoemaker et al. 1997).

In the wild, carnivores such as felids spend much of their active time hunting, but captivity restricts their ability to express normal hunting behaviors because food is provided in an easily obtainable and digestible form on a predictable schedule (Shepherdson et al. 1993). Additionally, felids' natural methods of hunting prey cannot be exercised due to the ethical and public concerns with live prey (Pitsko 2003). The lack of space in zoo environments makes it difficult to accommodate felids' tendency to range over wide geographic areas. Wide-ranging felids are accustomed to experiencing new local environments frequently; therefore, static zoo environments impede the exhibition of natural behaviors. Lions have some of the most expansive ranges of the large felids, a factor that is thought to contribute to a lack of reproductive success and high infant mortality rates in captivity (Shoemaker et al. 1997; Clubb and Mason 2007).

Mallapur and Chellam (2002) studied the activity budgets of Indian leopards (*Panthera pardus*) in four different zoos. They found that the leopards displayed more stereotypic behaviors in their off-exhibit enclosures and more active behaviors in their on-exhibit enclosures. The on-exhibit enclosures were more structurally complex, and usually much larger than the off-exhibit enclosures. Animals became more sensitive to noises and other disturbances after long periods of time in the off-exhibit enclosures. Mallapur and Chellam (2002) believed the increase in stereotypic behavior could be due to a lack of sensory stimulation. Similarly, a study by Bashaw et al. (2007) recorded the activity budgets of captive lions and tigers in various enclosures. Lions displayed less stereotypic pacing and more naturalistic behaviors when housed in a complex exhibit than in their off-exhibit enclosures. Tigers showed less pacing when housed in the larger exhibit. Bashaw et al. (2007) concluded that the types of behaviors being displayed were related to the environments in which they were housed.

2.5.5 Enrichment in Felids

Felids require enrichment that elicits natural feline behaviors. Enrichment strategies for felids can vary from exposing animals to stimuli such as frozen zebra dung and piñatas, to providing exercise courses and chase games (Van Metter et al. 2008). Environmental enrichment may reduce stress levels in captive felids that often lead to a wide array of vascular diseases, diseases that are generally observed only in captivity (Munson 1993; Munson et al.1999).

Large felids have proven to be particularly resistant to the effects of enrichment due to their naturally complex behaviors (Mellen and Shepherdson 1997). Additionally, temperament and personality are known to play a role in a felid's response to environmental enrichment (Boissy et al. 2007). Different dispositions among individuals may lead to different behaviors and coping mechanisms in response to the same stimulus (Wielebnowski 1999). Individual differences in fearfulness in felids and its impact on captive well-being has also been the subject of several studies. A study that focused on cheetahs found that female cheetahs tended to have more fearful temperaments than males, and non-breeding cheetahs tended to be more fearful than breeding cheetahs (Wielebnowski 1999). The more fearful cheetahs tended to cope more poorly with the captive environment than those with less fearful temperaments, suggesting that the fearful individuals might require more hiding places in their exhibits (Wielebnowski 1999). Structural enrichment including dens and hiding spots would allow the animals to escape from potential stressors.

Mellen and Shepherdson (1997) found that felids quickly habituate to novel conditions, meaning that enrichment must be constantly altered and reintroduced in order to be effective in encouraging more natural behaviors. Studies have shown that different felid species may vary in their reactions to enrichment activities, and slight environmental changes may alter specific biological functions, such as eating patterns and reproductive hormone secretion (Skibiel et al. 2007; Clubb and Mason 2007).

There are also interspecific and intraspecific differences in how animals respond to enrichment. Van Metter et al. (2008) found that using frozen blood balls, fresh zebra dung, scented squash, and cardboard boxes had a substantially greater beneficial effect on

African lions than on the Sumatran tigers. The African lions exhibited more active behaviors and slept less than the Sumatran tigers during the enrichment trials. However, the researchers also found that both species did not habituate to the enrichment over the trial period, indicating similarities in the responses of the two species. Moreira et al. (2007) found that the reproductive functions of female tigrinas and margays differed in response to environmental changes and presence of enrichment. Although increased corticoid concentrations and decreased reproductive function was seen in both species when housed in the small, barren enclosures, only the stress response of the tigrinas decreased when enrichment was added to the small enclosures, shown by the return of corticoid concentrations to baseline levels. Unlike the tigrinas, the enrichment did not elicit a decrease in corticoid levels in the margays, and their ovarian activity did not return to normal.

2.5.6 Generalization between Felids

Due to the small number of felids in zoo environments, felid enrichment experiments commonly use several species of felids to increase sample size (Skibiel et al. 2007). Because many felid species have similar physiologies and behavioral characteristics, similar enrichment strategies can be used for different species (Skibiel et al. 2007), which allows for some generalization between species. Studies have shown that identical enrichment items stimulate comparable responses in different species. A study on the effectiveness of different scent enrichments at the Montgomery Zoo used six different species of felids (Skibiel et al. 2007). Though the study found variations in the amount of response to the scent between species, the overall trend showed an increase in active behaviors and a decrease in stereotypic pacing (Skibiel et al. 2007). Bashaw et al.

(2003) showed that feeding enrichment, such as bones or fish, effectively reduced stereotypic behavior and increased activity levels in both Sumatran tigers and African lions. Other studies, such as the Van Metter et al. (2008) study comparing Sumatran tigers and African lions, have shown some variation due to age, sex, and species, but none of these were statistically significant due to small sample sizes. However, because the overall trends seem to be similar in different species, many enrichment studies generalize across species.

2.6 Mammalian Responses to Stress

Reeder and Kramer (2005) define stress as a “state in which homeostasis is lost.” A stressor can be physical, such as extreme temperatures or an injury to the animal; psychological, which can be any stimulus that causes an animal to become frightened, anxious, excited, or agitated; or a combination of physical and psychological. However, due to individual differences in animals, what may be a stressor to one may not be a stressor to another. In fact, what one individual considers stressful may change over time due to age, changes in the environment, or reproductive condition. The animal’s behavioral and hormonal responses to stress are designed to return the animal to homeostasis (Reeder and Kramer 2005).

2.6.1 Behavioral Response

An animal will alter its behavior to cope with a stressor. It will cease any behavior which can be delayed (such as eating) to instead engage in behaviors that will alleviate their immediate stress (Reeder and Kramer 2005). For instance, if an animal is faced with an intruder to its environment, its attention will be redirected. Instead of focusing on its current activity, the animal may respond with aggression, or it may flee or hide from the intruder. Other possible effects on an animal's behavior due to stress include altered cognition and attention span, increased awareness, altered sensory threshold, sharpened memory, stress-induced analgesia, and suppression of feeding or reproductive behavior (Reeder and Kramer 2005).

It has also been shown that an animal's body experiences stress in anticipation of a stressful event. For example, in the time shortly before waking, the body uses stress to provide the animal with the energy it needs for necessary behaviors such as hunting. When the animal is aroused, it will respond to the stress with the appropriate behavior (Reeder and Kramer 2005).

However, there are times when an animal is incapable of carrying out its desired behavior. When an animal is in captivity, its behavioral options become limited. It may feel compelled to hunt, run, or hide, but be unable to do so due in the captive environment. Instead of performing the primary behavior, the animal may perform a stereotypy or abnormal behavior (Mallapur and Chellam 2002).

2.6.2 Hormonal Response

The typical mammalian stress response was characterized by Selye (1936) and termed the General Adaptation Syndrome. The first stage of response is alarm, when the animal recognizes a stimulus as potentially "threatening." The alarm stage is also known as the "fight or flight" reaction. "Fight or flight" is characterized by the production of the hormones adrenaline and cortisol. Elevated concentrations of cortisol and adrenaline elicit an increase in the sympathetic nervous system activity. This reaction allows the animal to flee from the stressor, or to confront the stressor until the threat is neutralized.

The stressor causes the hypothalamus to produce ACTH (adrenocorticotropin hormone) which travels through the bloodstream to the adrenal cortex, stimulating the release of cortisol into the bloodstream. The downstream effects of cortisol suppress non-vital functions in the animal, and direct that energy toward dealing with the stressor. The stressor may manifest in increased cellular metabolism to make energy more available or increased respiration rate. Meanwhile digestion, reproduction, and maintenance of the immune system are temporarily decreased until the stressor is eliminated.

In the presence of a natural stressor where the animal is able to escape, the alarm stage would be the end of the stress response and suppressed non-vital functions would resume. For example, when a white-tailed deer encounters a hunter in the woods, the deer recognizes the human's presence as a threat. To respond the deer runs in the opposite direction as fast as possible. The deer can escape from the perceived stressor and neutralize the threat. Though we refer to these events as "stressful," it should be noted that this is a natural response from the body, as contrasted by "distress" where the animal begins to experience detrimental physical effects from exposure to stressors (Wielebnowski 2003). Such an event may occur when the animal is unable to escape a persistent stressor. This is referred to as the resistance stage of the stress response. When an animal is consistently stressed, as in the resistance stage, these non-vital functions are suppressed indefinitely or until the stressor is removed. In the long term, elevated stress manifests in reduced fecundity, a compromised immune system, and lack of appetite. These effects compound in the animal and lead to gradual weakening until the exhaustion stage is reached. When exhaustion is reached, the animal can no longer cope with the consequences of elevated levels of cortisol and eventually dies due to malnutrition or secondary infection (Figure 2.12) (Selye 1936).

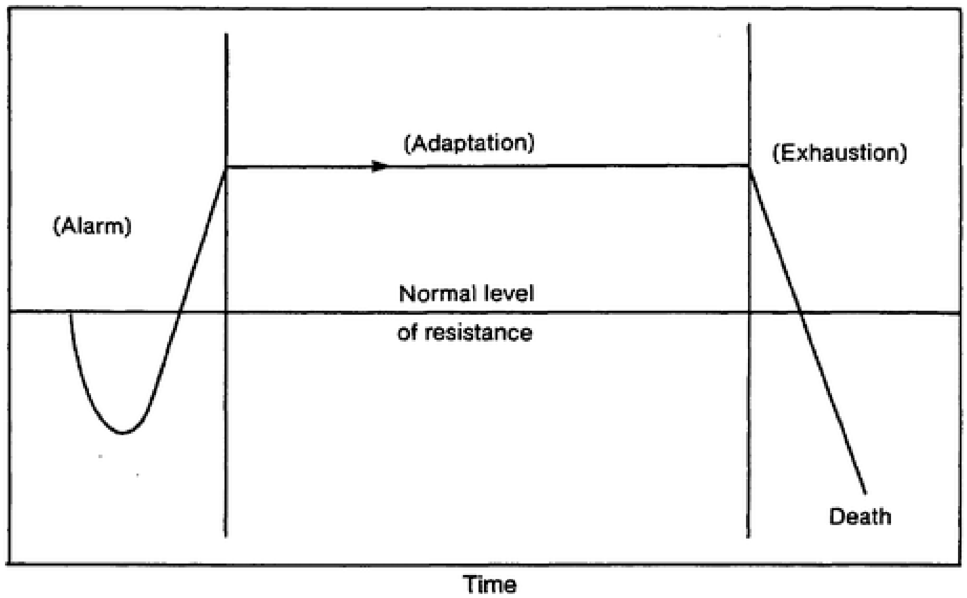

Figure 2.12 Selye's General Adaptation Syndrome (Smith 1987).

Though high levels of persistent stress are undesirable, low levels of stress have been shown to improve well-being of an animal. When an animal experiences normal levels of stress, the hormone cascade elicits the production of additional substances along with stress hormones. The substances include extracellular gases and neurotransmitters which can bolster the immune system and may help combat disease when produced in low concentrations associated with normal stress (Mattson 2008). This process is called hormesis and may be brought about by exercise.

However, zoo animals often experience persistent stressors from which they are unable to escape. Thus animals in zoos may experience the resistance stage of Selye's General Adaptation Syndrome, manifesting in reduced fecundity and illness. As the objectives of a zoo are to educate the public and establish a successful breeding program, cortisol resistance poses a real threat to the success of these goals.

2.7 Hormone Monitoring through Corticoid Analysis

Fecal corticoid measurements are often used to monitor changes in animals' stress levels. Because of the physiological connection to stress and the benefit of being non-invasive, fecal corticoid measurements have been implemented in many studies evaluating the effects of enrichment on an animal's level of stress (Wielebnowski 1999). Corticoids are part of the hormonal response of the physiological stress reaction. They are produced by the body when a stressor is perceived, metabolized when no longer needed, and are excreted as waste in the feces. Therefore, measurements of hormonal levels in fecal samples can provide useful information about the activity of the hypothalamic-pituitary-adrenal axis (Wielebnowski 2003).

Because of the ability to monitor stress responses practically and non-invasively, fecal corticoids are preferred over other methods such as blood and urine sampling. Though blood samples show instantaneous changes of various hormones in response to stressors, the simple act of collecting samples can actually increase stress in the animal and create a confounding variable (Keay et al. 2006). Urinary corticoid measurements, like fecal corticoid analysis, are also non-invasive, reducing the possibility of inducing stress through sample collection. However, the practicality of this approach for felids is limited because they tend to spray when they void urine. Thus collecting uncontaminated urine in quantities sufficient for analysis becomes difficult. Fecal glucocorticoid measurements reflect not only the glucocorticoids, but also the metabolized versions of the hormone. This inclusive collection of metabolites better encapsulates the amount of glucocorticoid secreted by the animal. Depending upon how frequently the animal defecates, a fecal pellet may contain the glucocorticoids produced in a single day or multiple days. Because the cat has to metabolize and excrete the cortisol, there is a "lag" in when the stressful event occurred and when the hormones are excreted (Hodges et al. 2010). However, fecal corticoid levels provide a daily (or almost daily) profile of the animal's stress level. This profile may be plotted over weeks or months, and when the concentration remains elevated the subject may be experiencing chronic stress (Keay et al. 2006). A study of corticoid metabolism in domestic cats also showed that fecal corticoids better reflected the actual concentrations of stress hormones within the cat's bloodstream than urinary corticoid measurements (Graham and Brown 1996).

Not all stressful events are mediated through the creation of cortisol to elicit a response. Prolactin and catecholamines may be vital portions of the stress response, and cannot be measured in fecal pellets. However, in the case of felids, the feces provide the best opportunity to monitor stress hormones since cats excrete almost all of their cortisol metabolites in their feces. Although the profile may not be complete, feces are the best means to obtain data non-invasively (Hodges et al. 2010).

Fecal corticoids are measured with immunoassays, and all assays must be validated before use to ensure that the hormones of interest are measured accurately. There are two varieties of assay commonly used: radioimmunoassay (RIA) and enzyme immunoassay (EIA). The former utilizes radioactive isotopes to label the hormone of interest, whereas the latter uses an antibody bound to an enzyme to emit a visual signal when the antibody binds to the hormone of interest. Most zoos utilize the EIA because it does not require the radioactivity of the RIA, thus making it safer for use in zoological institutions that would otherwise be restricted in their ability to use such substances (Young et al. 2004).

It is vital to the integrity of any study that all assays and extraction techniques are sensitive enough to determine fluctuations within the animal's cortisol level that may be of biological significance (Touma and Palme 2005). Thus, before a particular assay can be used, it must be validated. Validation is comprised of two major aspects: analytical and physiological validity. Analytical validity refers to the sensitive of the assay to different levels of hormones, as well as the general accuracy and precision of the assay (Palme 2005). This can be established by using the assay to analyze several different, known dilutions of the relevant hormone, and ensuring that the assay reflects the correct level of the hormone.

Physiological validity refers to the assay being responsive to actual changes in circulating hormone levels. Establishing physiological validity involves pharmacologically inducing changes in corticoid levels in the bloodstream and assessing if the assay reflects those changes (Touma and Palme 2005). This is done by taking samples before and after events known to induce glucocorticoid change (e.g. exogenous administration of ACTH, stressful events such as veterinary exams) and seeing if the assay detects a marked change in hormone levels as a result. Measuring hormonal changes can be complicated by differences between species, sexes and life history stages, making it necessary to validate assays with large sample sizes to control for such variation (Palme 2005).

Though fecal corticoid levels can provide important data, the results cannot serve as the sole basis for conclusions regarding an animal's stress level. This is due to the fact that corticoid has a natural circadian rhythm that fluctuates throughout the course of the day and corticoid levels vary widely among individual animals (Hodges et al. 2010). Measurement of fecal corticoid levels is an average measurement of this fluctuation (Keay et al. 2006). There are many factors within the nervous system, including regulation of the neurotransmitters involved in the stress response that may alter corticoid levels in the feces. Additionally, normal corticoid ranges vary between individuals. High corticoid levels typically indicate the presence of stress, but it is often difficult to differentiate between eustress, which might be the result of increased activity or interaction with other animals, and distress, which may result from anxiety and boredom (Wielebnowski 1999).

Chapter 3: 2011 National Enrichment Practices Survey

3.1 Overview

We designed the *2011 National Enrichment Practices Survey* (see Appendix A) to characterize current enrichment practices for lions and tigers and to provide context for our subsequent study. The survey was broadly distributed to zoos throughout the United States and asked for information regarding husbandry schedules, enrichment practices, and limitations to implementing enrichment.

3.2 Survey Methods

3.2.1 Questionnaire

The survey was administered as a Google Documents online form with ten required questions and thirteen optional questions. The questions were either short answer or multiple choice. A draft of the survey was sent to curatorial staff and felid research scientists at the Smithsonian National Zoological Park (Washington, DC) and Brookfield Zoo (Chicago, IL) for feedback, and their suggestions were incorporated into the final version. We focused on two species (lions and tigers) for two main reasons. Lions and tigers are particularly numerous at zoos, so we anticipated that choosing these species would give us a robust sample size. Also, our initial endocrine analysis phase focused on lions and tigers because those were the two large felid species maintained at our collaborating institution, the National Zoological Park.

The survey consisted of three sections. In the first section, respondents were asked how many lions and tigers were maintained at their institution, how long the animals were on exhibit each day, and details about the animals' enclosures, such as what percentage of the enclosure was composed of natural substrate. There was also a free response area for a more detailed description of enclosure size and design.

The second section focused on the types of enrichment being used for lions and tigers. Respondents were provided with a list of enrichment techniques (derived from the literature) and asked which they used. Respondents were also given a free response area to provide additional details concerning use of enrichment in general, and specifically the implementation of scent enrichment. We also asked how often enrichment was implemented and the size of the institution's monthly enrichment budget. This section also asked whether there was perceived limitations to the institution's ability to provide enrichment and the nature of those limitations.

The final section of the survey consisted of questions to characterize the responding zoos more fully. We asked respondents to provide the total number of species exhibited, size of the institution (in acres), number of employees involved in animal care, number of employees involved in the care of felids, daily number of visitors during the peak season, zoo ownership (private or public), and the type of accreditation the zoo held, if any.

3.2.2 Survey Distribution

To obtain a comprehensive picture of enrichment in zoos nationwide, we sent emails to zoos, providing the URL for the online survey, explaining its purpose, and requesting that a representative of the institution provide a response. The mailing list was developed by collecting contact information for zoo employees involved with felid care from the AZA Membership Directory. Because only a subset of zoological institutions is AZA-accredited, we also conducted a comprehensive Internet search to identify zoos that did not hold AZA accreditation. We sent the survey to 212 zoos on February 6, 2011, with a deadline for responses one week later. Many of these emails failed to reach their intended recipients, so we searched for other individuals to contact within those zoos and sent the email to them. After approximately 20 institutions had responded, we reviewed the geographic distribution of those responses and identified geographic areas that were underrepresented. We then made a focused attempt to contact individuals at institutions in those areas. Finally, we re-sent the survey specifically to institutions that were not AZA accredited because those types of institutions were underrepresented in our sample. Our final response rate was 19%, with 26 of the 50 states represented (Figure 3.1).

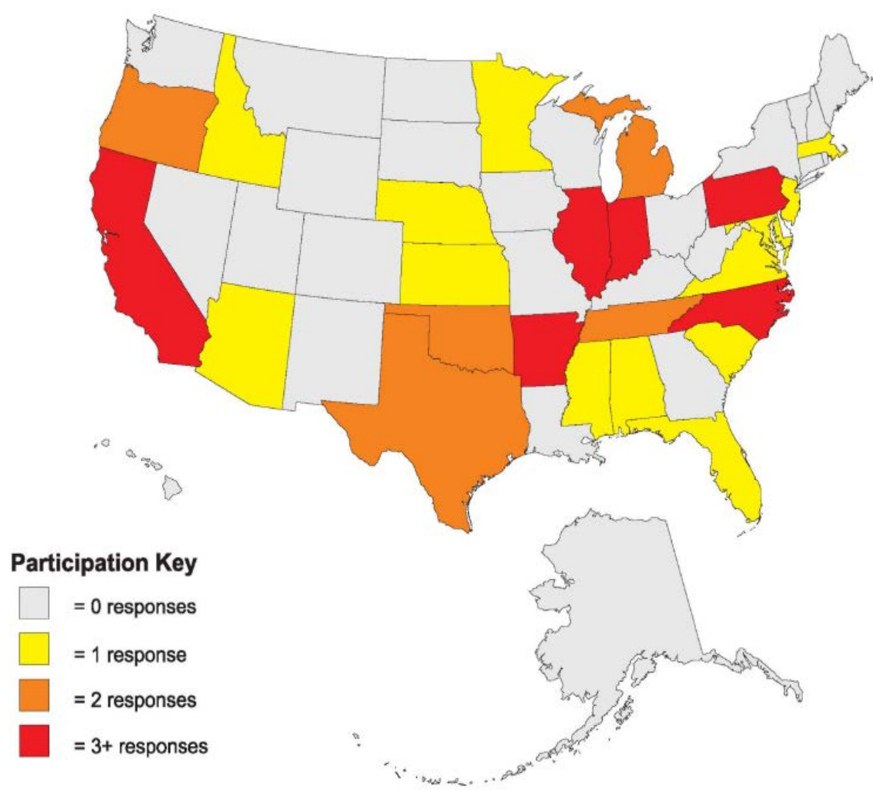

Figure 3.1 Geographic representation of zoos that responded to the 2011 National Enrichment Practices Survey.

3.2.3 Data Analysis

We employed descriptive statistics to characterize the zoos and their husbandry practices. We then used analytical statistics to test for differences between zoos in how enrichment was used and the types of limitations on the use of enrichment. Thirty-nine zoos responded to the survey, for a response rate of 19%; however, not all respondents answered every question, resulting in smaller samples sizes for some questions.

We used Fisher's exact test to examine differences between subgroups of zoos in how enrichment was used. We used Spearman rank correlations to examine the degree of agreement between subgroups of zoos in the factors that influenced their ability to employ enrichment.

3.3 Survey Results

3.3.1 Descriptive Statistics

3.3.1.1 Enclosures

Most zoos reported that their lion and tiger exhibits were composed mainly of natural substrates, including grass, dirt, and plants (Figure 3.2). Many respondents reported that the enclosures had some variety of rocks, a water structure, trees and bushes, and occasionally different levels or caves for the animals to access.

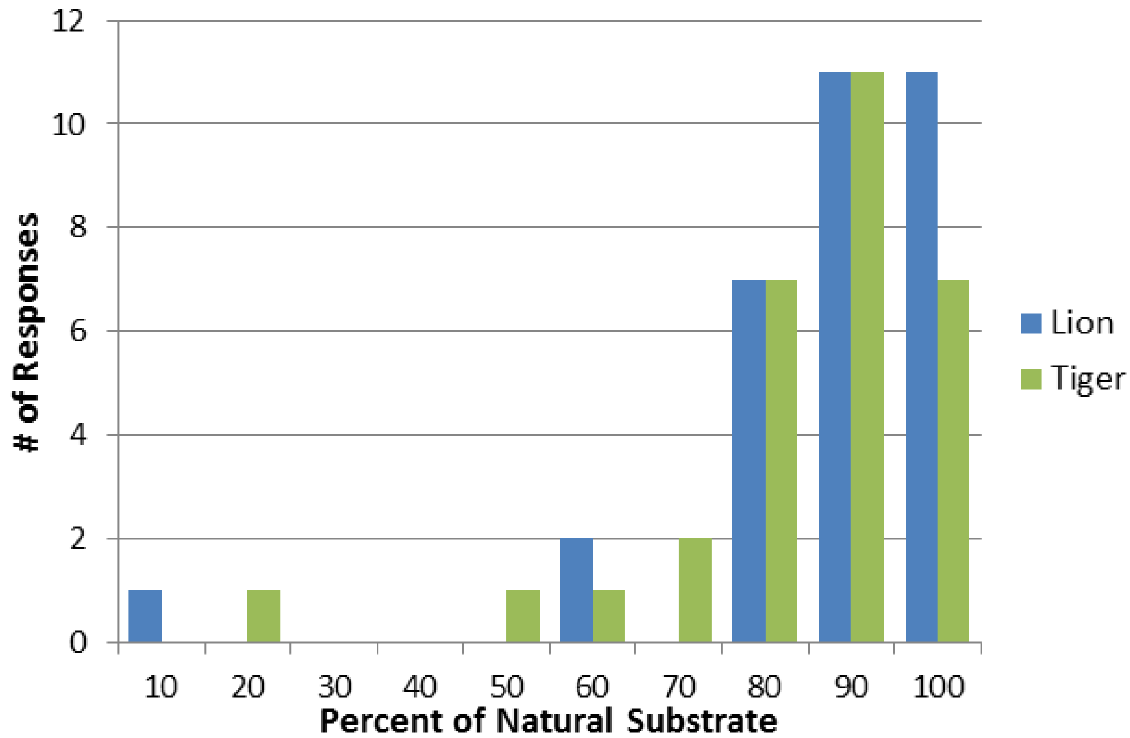

Figure 3.2 Percent of natural substrate in the lion and tiger enclosures.

3.3.1.2 Exhibit Time

For both lions and tigers, time on exhibit averaged approximately 13-14 hours per day, however variation between zoos was high (Figure 3.3). For both species, time on exhibit ranged from 0-1 hour up to 24 hours per day. There were also seasonal differences, as some animals spent more time on exhibit during the summer months than in winter.

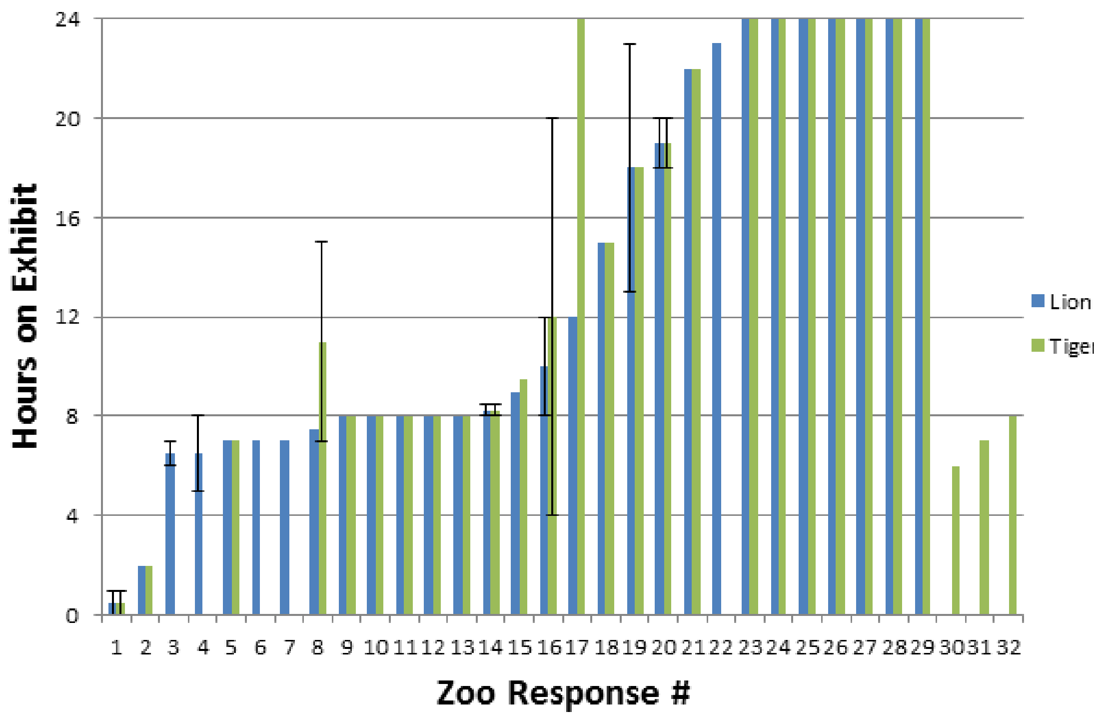

Figure 3.3 Average number of hours on exhibit for lions and tigers. Error bars indicate a range of hours reported in the survey.

3.3.1.3 Enrichment Use

To determine the types of enrichment that zoos used for lions and tigers, a list of 18 options gleaned from the literature was provided. Respondents could select as many types of enrichment as they used. The most used enrichment (in terms of the percentage of zoos reporting its use) was perfume, with 100% of respondents indicating its use (Figure 3.4). Mirrors were the least common form of enrichment, used by only 23% of zoos. On analyzing the survey, we discovered that there was a typographical error in one of the options (the option read “Animal Scenes” rather than “Animal Scents”). While the number of respondents selecting this option was low, its relative prevalence was difficult to interpret because we could not be sure how it was interpreted by respondents.

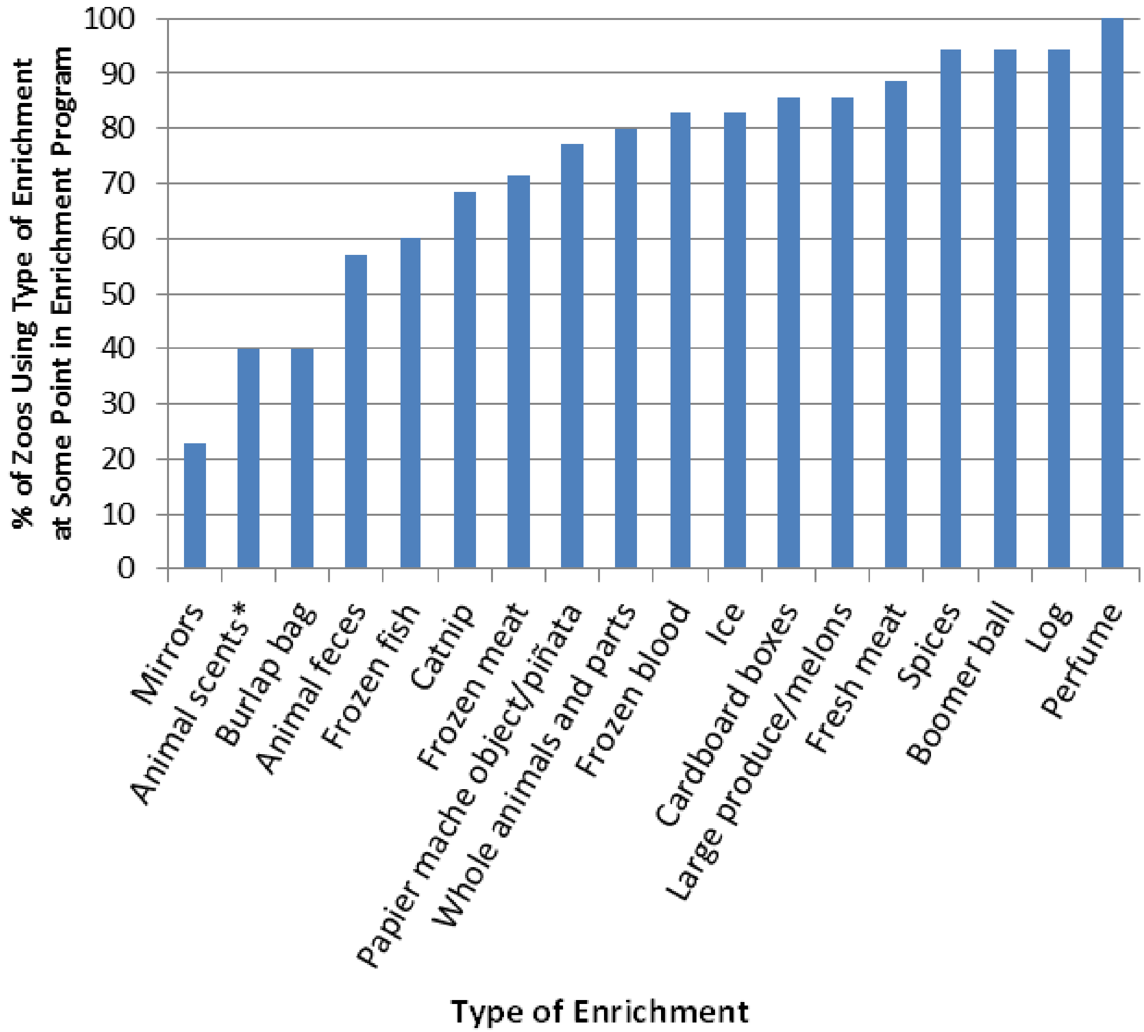

Figure 3.4 Frequency of different enrichment practices. *Typographical error in survey listed this option as "Animal scenes."

Of the 37 respondents who reported on the frequency of enrichment use for lions and tigers, over half (57%) indicated that they used some form of enrichment daily, while 35% used enrichment multiple times per week. In total, 97% of respondents used some form of enrichment at least once a week for their lions and tigers. The various frequencies of enrichment use are shown in Figure 3.5.

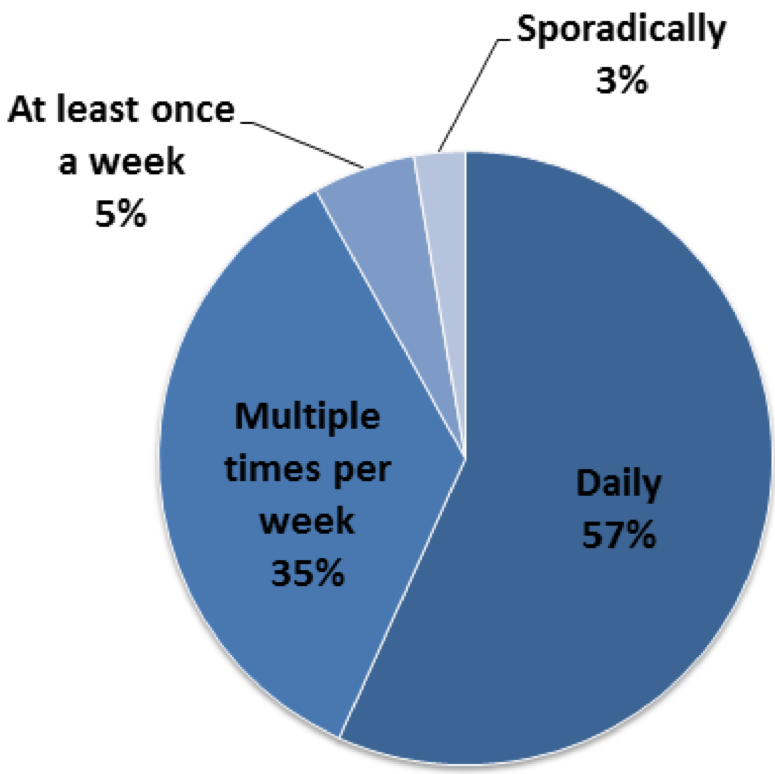

Figure 3.5 Frequency of enrichment use.

Most respondents (78%) reported spending $0 to $50 on enrichment each month. Many opted to provide additional information on how they acquired and implemented their enrichment supplies. Zoos frequently used donated items for enrichment, as well as using seasonal items (e.g. pumpkins and Christmas trees) that were easily acquired through donations.

3.3.1.4 Limitations on Enrichment Use

The majority of respondents, 72%, reported feeling that their ability to provide enrichment was limited. Respondents were given seven options for limiting factors and were asked to select all that applied. The most frequently selected limiting factors were funding (49%) and time (49%) (Figure 3.6).

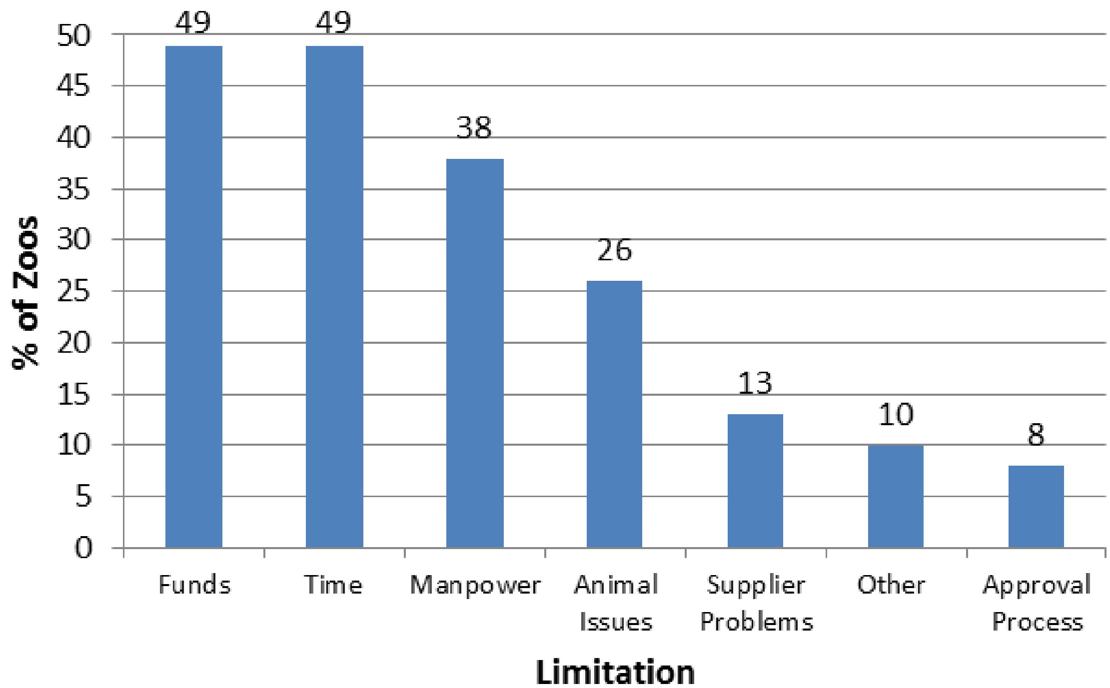

Figure 3.6 Factors limiting use of enrichment.

3.3.2 Analytical Statistics

3.3.2.1 Defining Size

Our survey contained four numerical measures that were indicative of institution size: number of acres, number of species, total number of staff, and average daily attendance during peak season. For each measure, we ranked the institutions in increasing order of their numerical responses. We then divided zoos into two groups, corresponding to those above and below the median for each measure. We then assigned each institution a numerical score of 1 if the institution's response was in the lower group and 2 if the response was in the higher group. We the averaged the scores for each institution across all four variables to create a variable summarizing the institution's size relative to other institutions in the sample, Institutions with average scores below the overall median

(N=19) were characterized as small, while institutions with average scores at or above the median (N=20) were characterized as large.

3.3.2.2 Characterizing Responding Zoos

AZA is the predominant accrediting body in the zoo field and has the most stringent accreditation rules, so we divided the institutions into AZA-accredited (N=28) and non-AZA accredited institutions. (N=11) The final dimension we used to characterize zoos was whether they were publicly (N=26) or privately (N=13) owned.

3.3.2.3 Frequency of Enrichment

Thirty-seven of thirty-nine institutions responded to this question. Twenty-one of those responding (57%) provided enrichment daily, while 16 (35%) provided enrichment less often. AZA-accredited institutions were significantly more likely to provide enrichment daily than were non-AZA accredited institutions (Figure 3.7; Fisher's exact test, p=0.028). The frequency of providing enrichment did not differ between large and small institutions (Figure 3.8), or between publicly and privately owned zoos (Figure 3.9).

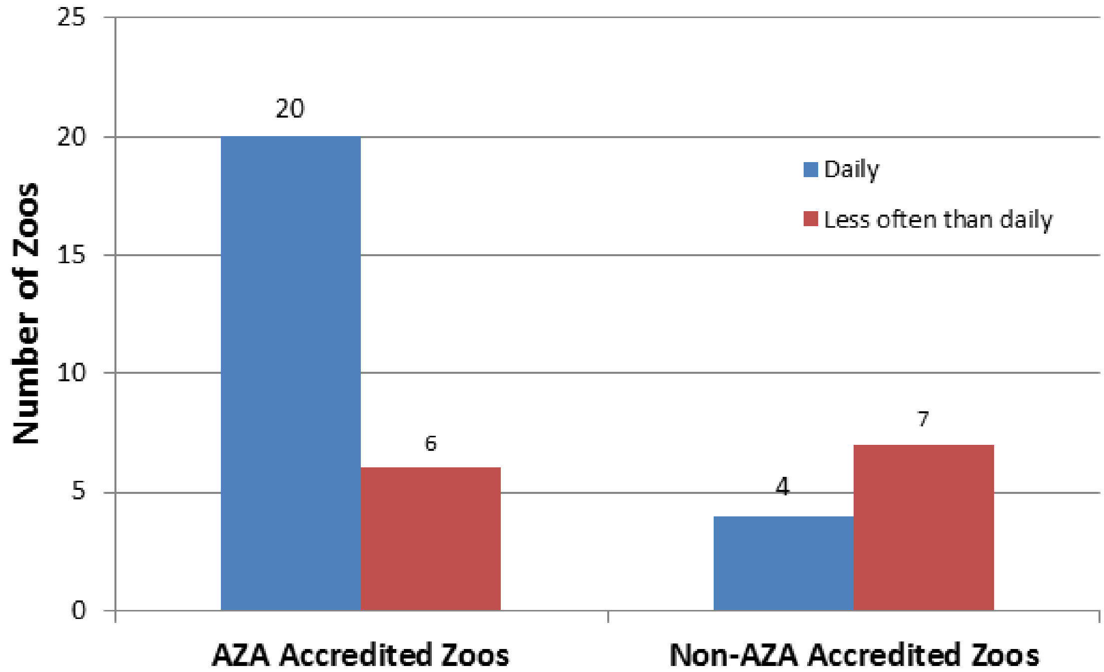

Figure 3.7 Frequency of enrichment usein relation to accreditation; Fisher's exact test, p=0.028 (2-tailed).

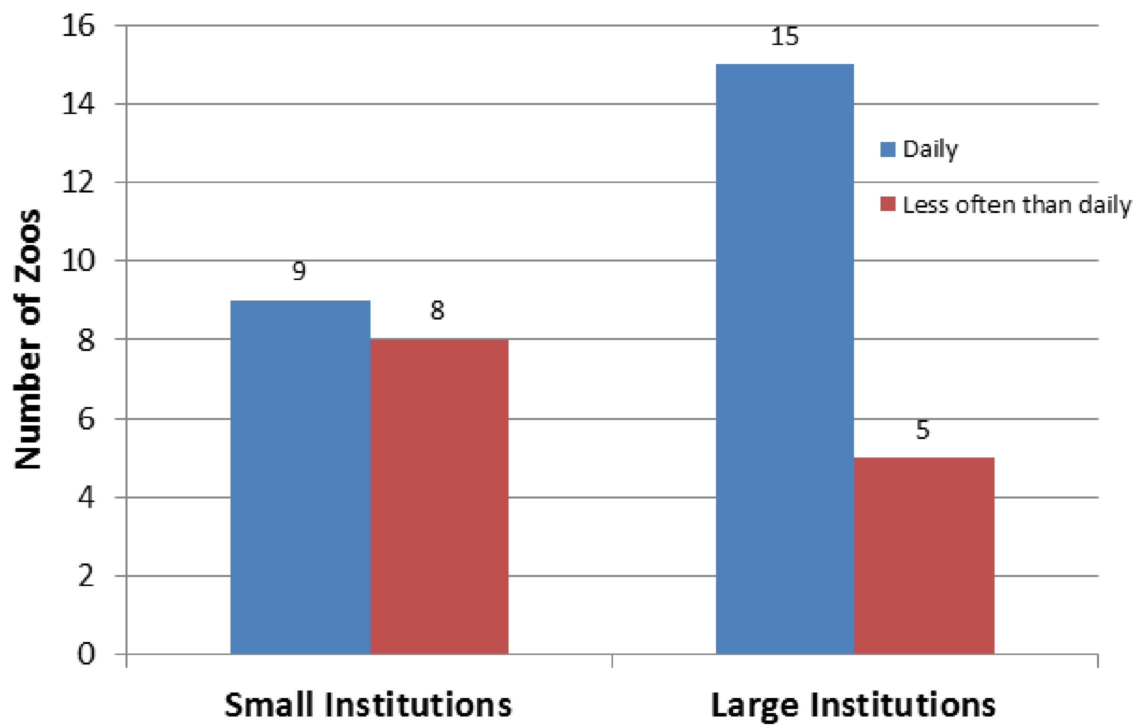

Figure 3.8 Frequency of enrichment use in relation to zoo size; Fisher's exact test, p=0.188 (2-tailed).

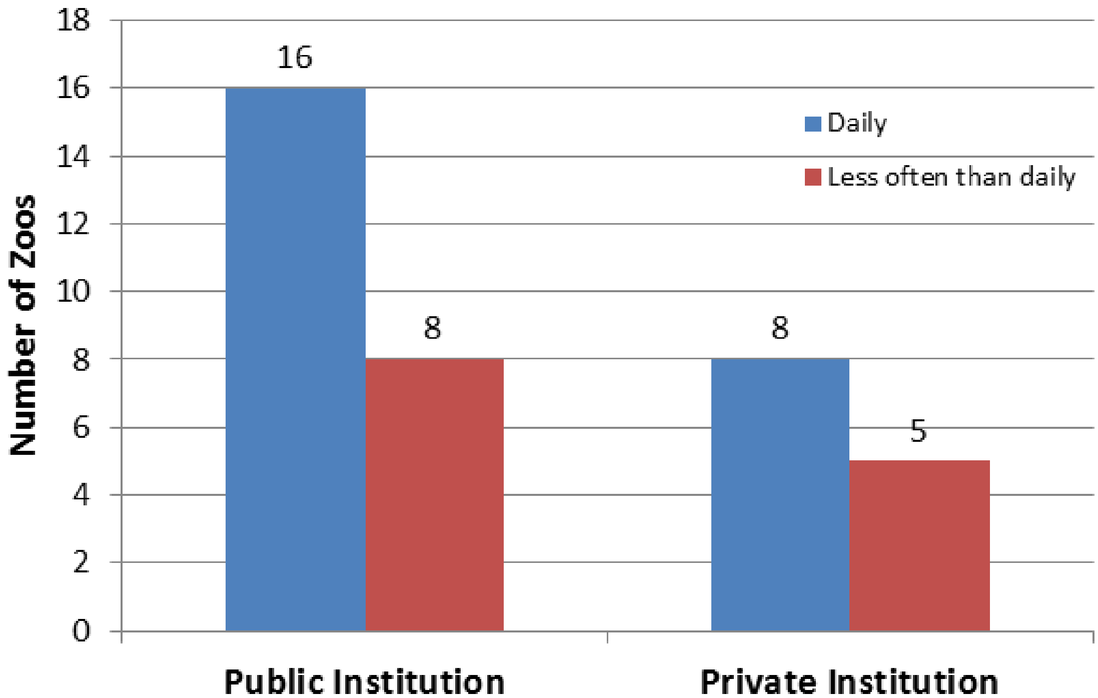

Figure 3.9 Frequency of enrichment use in relation to zoo ownership; Fisher's exact test, p=1.000 (2-tailed)

3.3.2.4 Perceived Limitations in the Use of Enrichment

Thirty-six of the 39 responding institutions replied to this question. Twenty-six of those responding (72%) indicated that they felt limited, while 10 (38%) did not feel limited. Whether an institution felt limited in their ability to provide enrichment was not related to the type of accreditation (AZA or other) (Figure 3.10), institution size (small or large) (Figure 3.11), or type of ownership (public vs. private) (Figure 3.12).

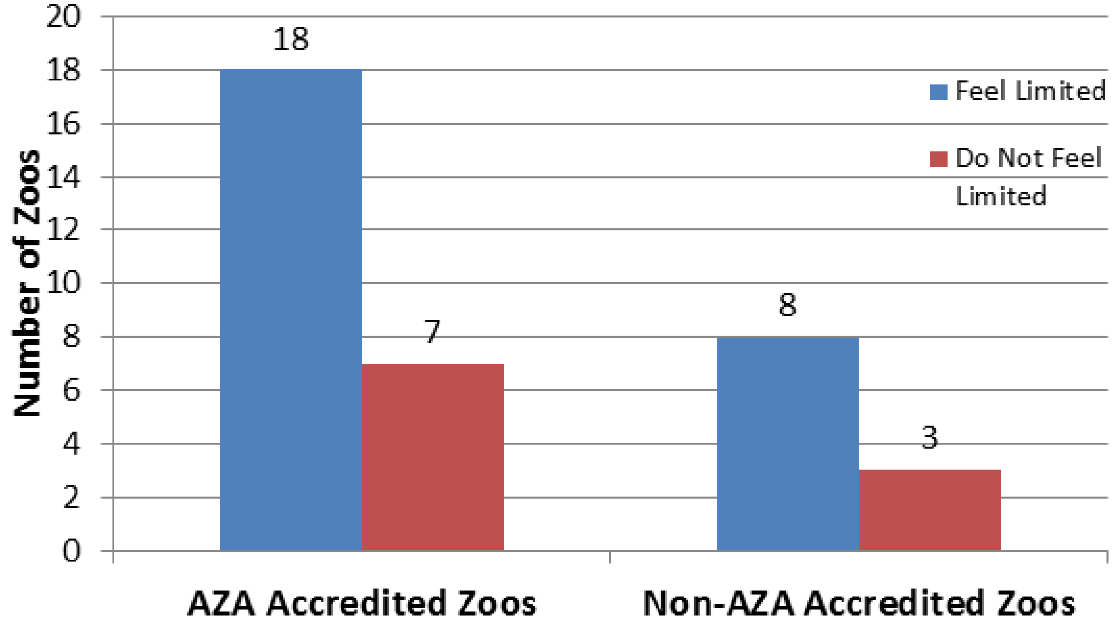

Figure 3.10 Perceptions of limitation in relation to accreditation; Fisher's exact test, p=1.000 (2-tailed).

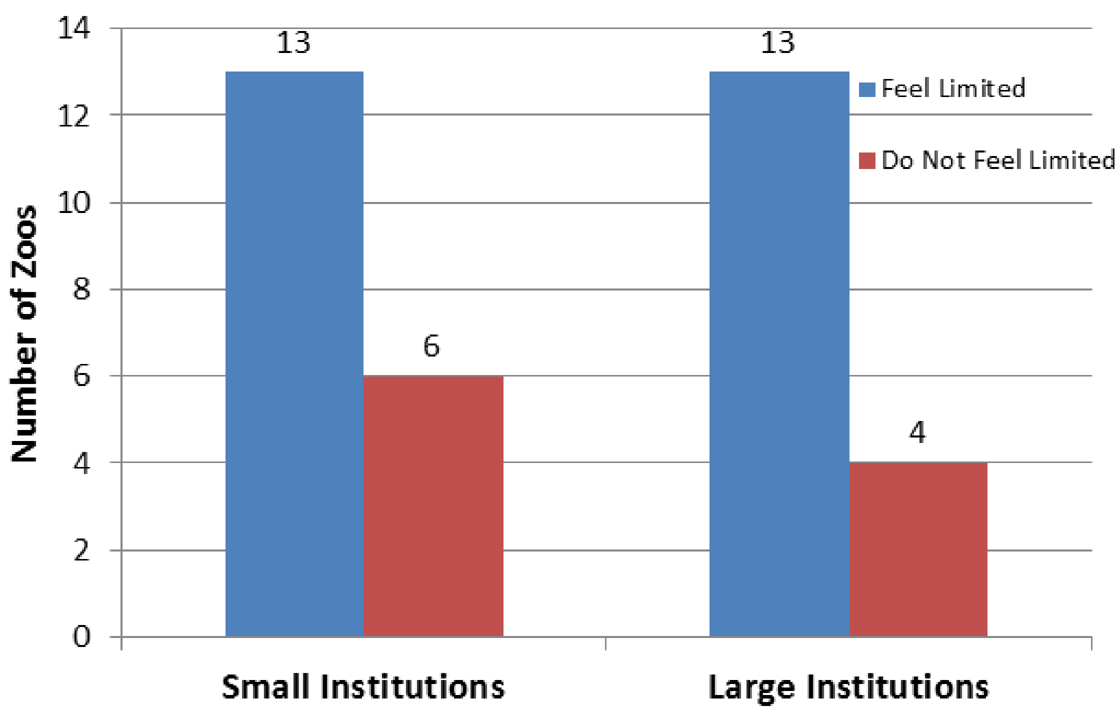

Figure 3.11 Perceptions of limitation in relation to zoo size; Fisher's exact test, p=0.717 (2-tailed).

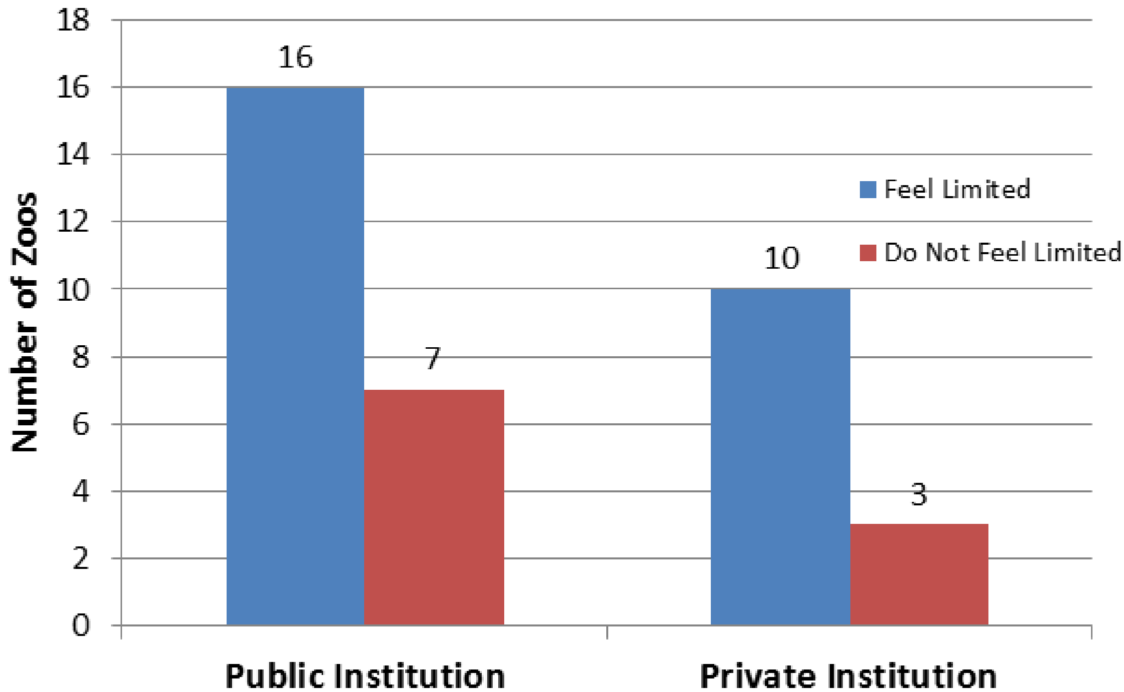

Figure 3.12 Perceptions of limitation in relation to zoo ownership; Fisher's exact test, $p=0.716$ (2-tailed).

3.3.2.5 Factors Limiting Use of Enrichment

Overall, zoos were in agreement regarding the factors that limted their use of enrichment. We found strong correlations between small and large institutions ($R_s=0.778$) (Table 3.1), public and private institutions ($R_s=0.771$) (Table 3.2), and AZA and non-AZA accredited institutions ($R_s=0.898$) (Table 3.3) in the relative importance of each of the limiting factors. Across all zoo dimensions considered in our analysis, funds, manpower, and time were determined to be the factors most limiting to zoos' enrichment use (F).

Factor	Number of small institutions	Number of large institutions
Funds	10 (53%)	9 (45%)
Manpower	10 (53%)	5 (25%)
Time	10 (53%)	8 (40%)
Animal issues	4 (21%)	6 (30%)
Suppliers	3 (16%)	2 (10%)
Approval process	2 (10%)	0 (0%)
Other	1 (5%)	3 (15%)

Table 3.1 Factors limiting enrichment based on zoo size; Spearman rank correlation Rs=0.778.

Factor	Number of public institutions	Number of private institutions
Funds	13 (50%)	6 (46%)
Time	10 (38%)	9 (69%)
Manpower	9 (35%)	6 (46%)
Animal issues	7 (27%)	3 (23%)
Suppliers	3 (12%)	2 (15%)
Other	2 (8%)	2 (15%)
Approval process	0 (0%)	3 (23%)

Table 3.2 Factors limiting enrichment in relation to zoo ownership; Spearman rank correlation Rs=0.771.

Factor	Number of small institutions	Number of large institutions
Funds	14 (50%)	5 (45%)
Time	11 (39%)	8 (73%)
Manpower	10 (36%)	5 (45%)
Animal issues	8 (29%)	2 (18%)
Suppliers	4 (14%)	1 (9%)
Other	3 (11%)	1 (9%)
Approval process	2 (7%)	1 (9%)

Table 3.3 Factors limiting enrichment in relation to accreditation; Spearman rank correlation Rs=0.898.

Chapter 4: Initial Enrichment Assessment

4.1 Overview

In the second phase of the project, we sought to determine whether there was a relationship between stress hormone levels and the type of enrichment an animal experienced. The study was conducted at the National Zoological Park (NZP) in Washington, DC. The zoo houses over 2,000 individual animals on their 163 acre campus. The "great cats" enclosure is surrounded by a moat and is a multi-layer structure with natural substrate and rocks (Figure 4.1 and Figure 4.2).

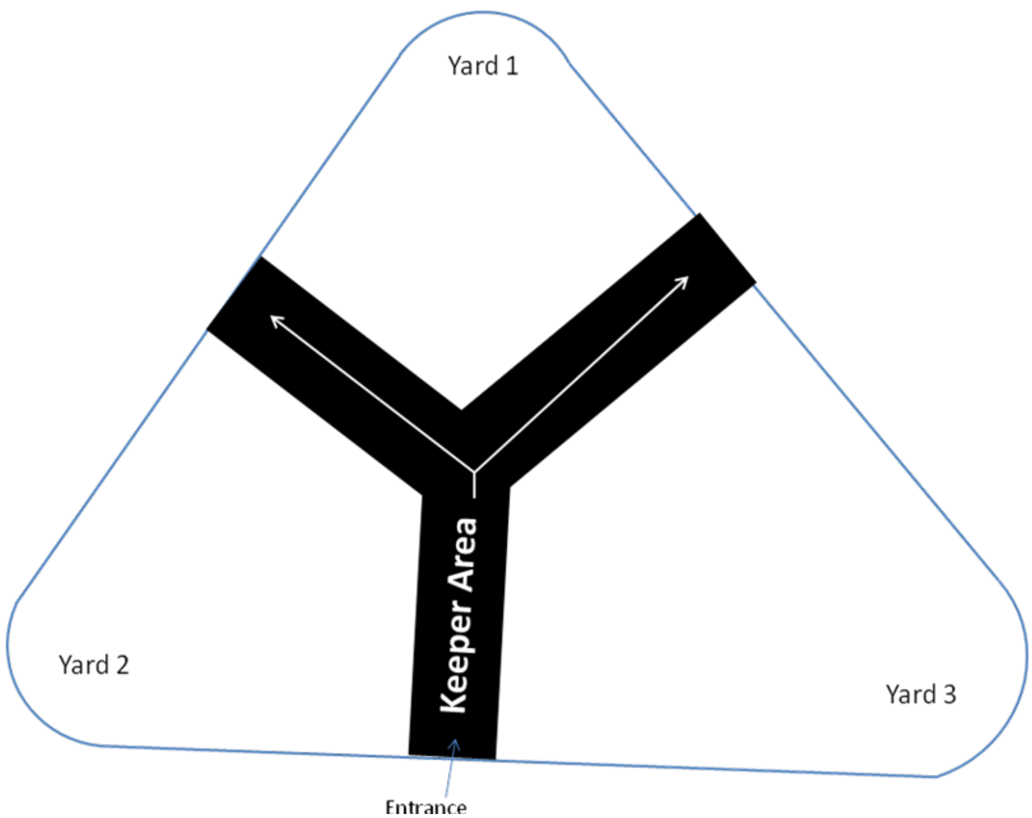

Figure 4.1 A simplified diagram of the lion and tiger enclosures at NZP.

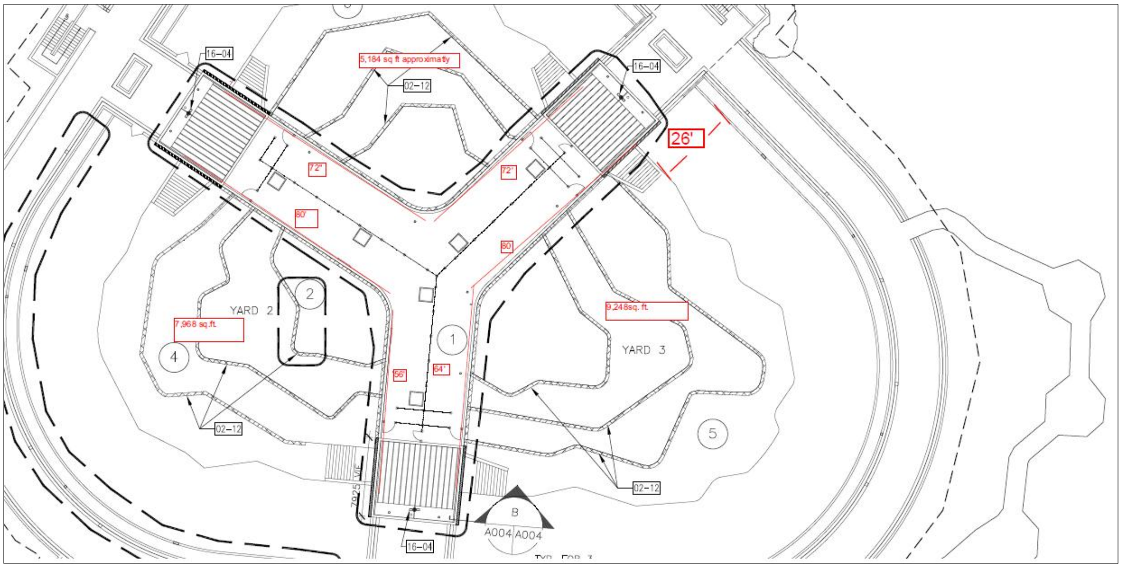

Figure 4.2 A schematic of the lion and tiger enclosures, including square footage at NZP.

4.2 Methods

During the summer and fall of 2010, zoo staff collected fecal samples from three lions and two tigers (Table 4.1) that were already on a predetermined enrichment schedule developed by the NZP animal care staff. Lions were given bones twice weekly, and frozen rabbits once weekly. The other four days of the week, keepers used their own discretion to select a single type of enrichment from various pre-approved enrichment techniques. Tigers were given a single enrichment per day at least five days per week, again selected by keepers from a list of pre-approved enrichment techniques. The type of enrichment was recorded by animal care staff in a daily log (see Appendix B); these records were saved for subsequent analysis.

Subject	Name	Species	Age	Sex
L1	Naba	*Panthera leo*	6.5	F
L2	Shera	*Panthera leo*	6	F
L3	Luke	*Panthera leo*	5	M
T1	Soyono	*Panthera tigris sumatrae*	17	F
T2	Gunther	*Panthera tigris sumatrae*	4	M

Table 4.1 List of subjects housed at the Smithsonian National Zoological Park during the study period (summer 2010).

At the beginning of the day, before animals were released into their viewing enclosures, animal care staff introduced the enrichment items into the enclosure. The animals were then released into the on-exhibit enclosures and were free to investigate or interact with the enrichment over the course of the day. During park closing, the animals were brought back into their night (off-exhibit) enclosures and keepers removed any remnants of the enrichment items.

Fecal samples were collected from the substrate when the enclosures were cleaned. Samples were collected both from each animal's' night enclosure and the on-exhibit enclosure. Different legumes (undigestible to felids) were routinely introduced into each lion's diet by animal care staff to enable individual identification of fecal samples. Freshly collected samples were placed in baggies, labeled with the identity of the animal and the date of collection, and stored at -20 ˚C for subsequent analysis. Samples were collected over a 130 day period (from 14 June 2010 to 22 October 2010). After all samples were collected, they were lyophilized at NZP facilities then sent to the Smithsonian Conservation Biology Institute (SCBI) Endocrine Research Lab in Front Royal, VA for hormone analysis (Figure 4.3).

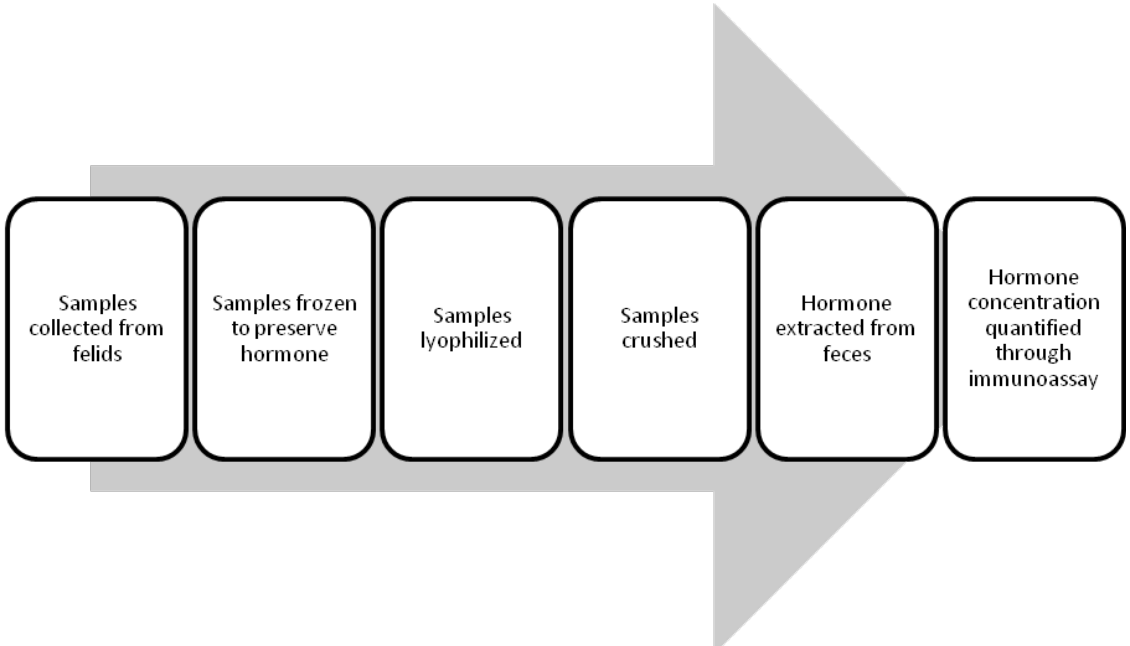

Figure 4.3 The process of preparing samples: fecal contributions were collected, frozen, dried, and crushed prior to their individual analysis.

Dried fecal samples were crushed and stored in labeled sample tubes. For extraction, 0.2 grams of each sample was weighed out and 5 mL of 90% ethanol was added. Extraction tubes were closed with rubber stoppers and placed on a multi-pulse vortex for 30 minutes. The vortexed samples were centrifuged at 1500 rpm for 20 minutes, and the supernatant was decanted into another set of labeled tubes. An additional 5 mL of 90% ethanol was added to the remaining fecal pellet in the original sample tubes, and the process of vortexing and centrifuging was repeated. The supernatant from the second round of centrifugation was added to the previous extract in the second set of sample tubes (Brown et al. 2008). The extract was dried and diluted as appropriate to achieve a dilution that fell within the standard curve for cortisol metabolite analysis (1:10 for lions, 1:20 for tigers).

An enzyme immunoassay previously validated for use in the species studied (Young 2004) was used to quantify the cortisol metabolite contained in each sample. The assay was performed in a 96 well plate. Wells were lined with an antibody that was sensitive to cortisol. 50 µL of standard concentrations of 3.9-1000 pg/well were included on the plate. 50 µL of extract occupied the remaining wells. 50 µL of cortisol-horseradish peroxidase (1:8500 dilution in assay buffer) was added to all wells. The plates were incubated for an hour and then rinsed 5 times with distilled water to remove excess extract. 100 µL of substrate buffer was added and the samples were again incubated for 10-15 minutes. The plates were read at 405 nm.

4.3 Results

The endocrine data were not normally distributed, so they were log-transformed for statistical analysis. Data were analyzed with an Analysis of Covariance (ANCOVA), with Subject and Day of Week as main effects and Day of Study as the covariate. The model included the interaction term of Subject*Day of Week. Univariate Analysis of Variance (ANOVA) was used to test the effect of different types of enrichment of cortisol levels. For both types of analyses, when significant effects were found Tukey's HSD was used to evaluate differences among individual treatments.

The overall model was a good fit for the log-transformed data ($F = 1.7721$, $df = 35, 178$, $p < 0.01$).There were significant differences in mean cortisol levels between the subjects (Table 4.2). T1 showed significantly higher levels of cortisol than the other subjects (Figure 4.4, Tukey’s HSD, $x = 2.540$, $p < 0.05$). There were no significant differences in cortisol production between the remaining subjects. There were no other significant effects. Cortisol levels did not vary systematically across the study period or by day of the week (Figure 4.5), and there was no significant interaction effect.

Source	DF	Sum of Squares	F Ratio	P
Weekday	6	0.1502	0.8082	0.5648
Cat	4	1.0092	8.1426	<0.0001
Day	1	0.0509	1.6429	0.2016
Weekday*Cat	24	0.8970	1.2062	0.2419
Error	178	5.5155	1.7721	0.0087

Table 4.2 Results from ANCOVA of effects.

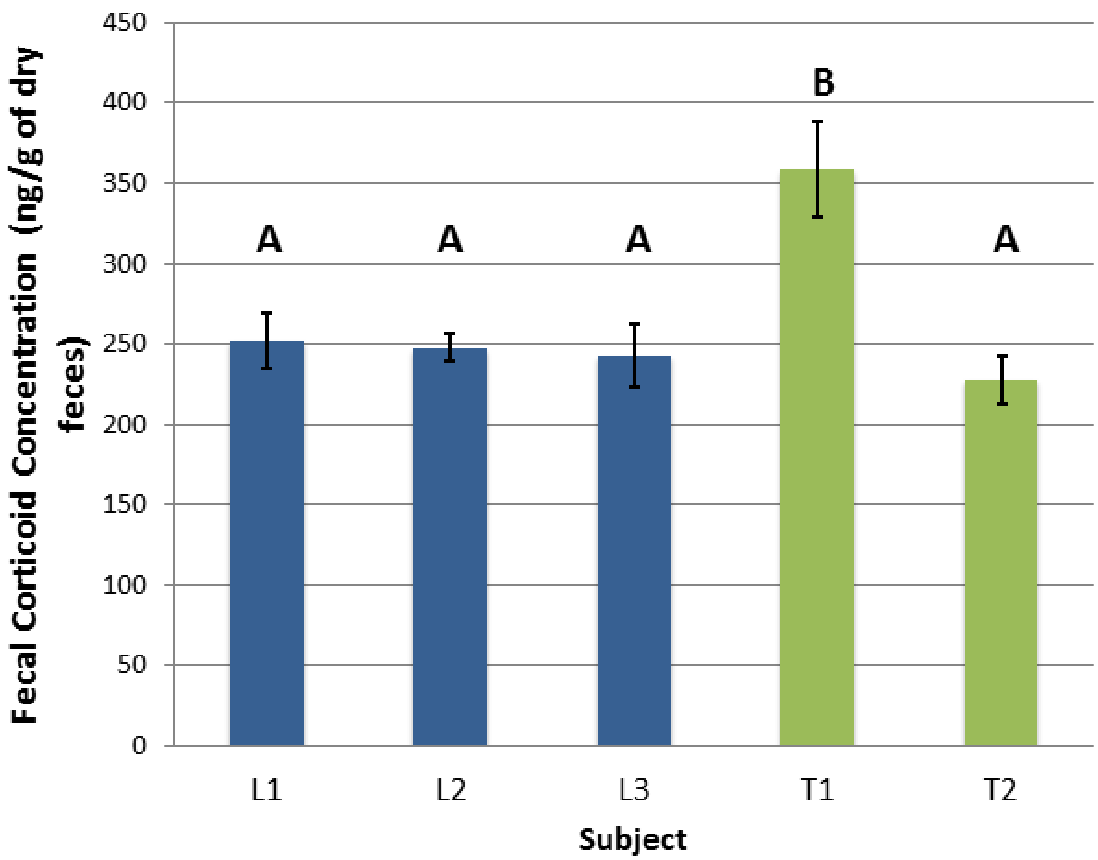

Figure 4.4 Fecal cortisol levels (mean $\pm$ SE) for three African lions and two Sumatran tigers maintained at the National Zoological Park.

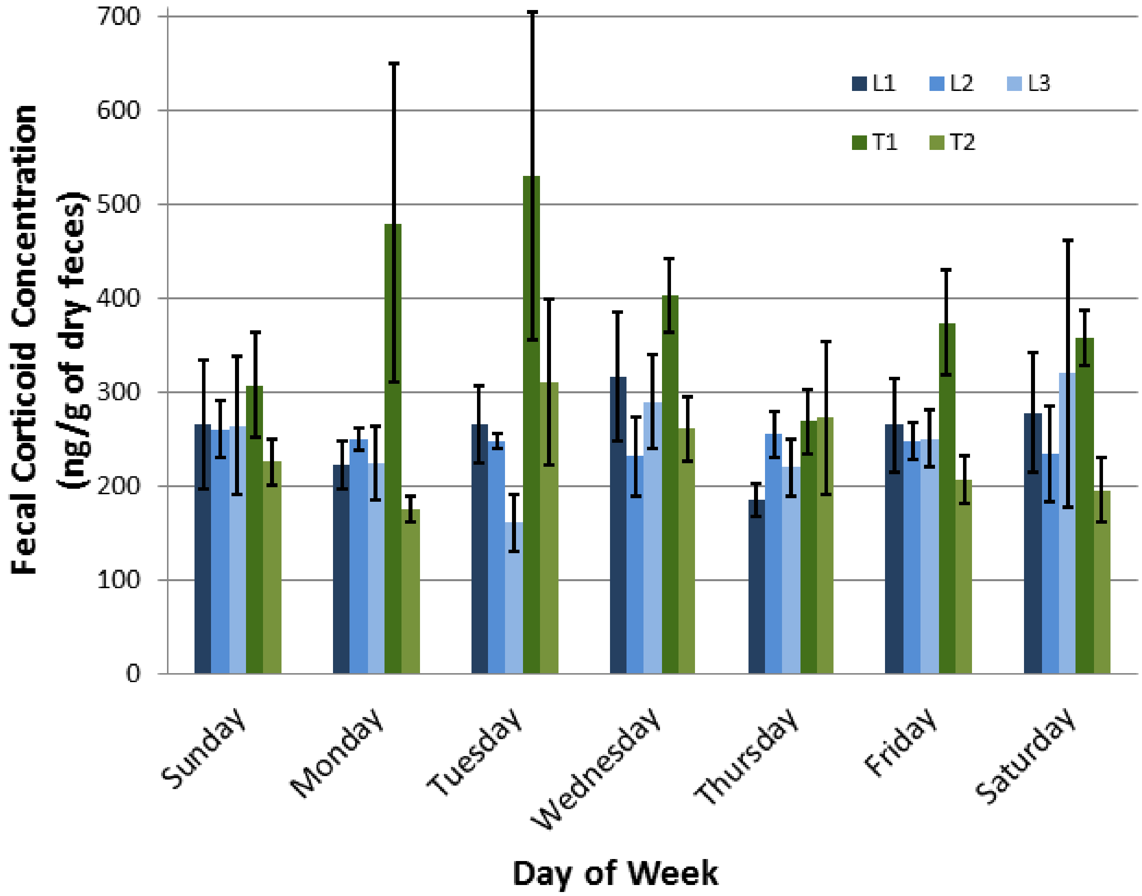

Figure 4.5 Fecal cortisol levels for subjects by day of the week (mean ± SE).

Because we found significant differences in cortisol levels between individual subjects and we did not have cortisol measurements for every type of enrichment for each subject, we conducted separate univariate ANOVAs to test for differences in cortisol levels associated with different types of enrichment. L2 was the only subject approaching significance (Table 4.3).

Subsequent analysis of the effect of enrichment type on cortisol levels for subject L2 showed that manipulative and feeding enrichments were associated with highest cortisol levels, which were significantly different from no enrichment (Figure 4.6). Cortisol levels associated with sensory and complex enrichment were intermediate and did not differ significantly from the others (Tukey's HSD).

Subject	df(model)	df(error)	F ratio	P
L1	3	38	0.8128	0.4947
L2	4	32	2.5905	0.0552
L3	3	28	0.1826	0.9073
T1	4	30	0.9021	0.4751
T2	3	32	0.4017	0.7527

Table 4.3 Results from ANOVA of different types of enrichment.

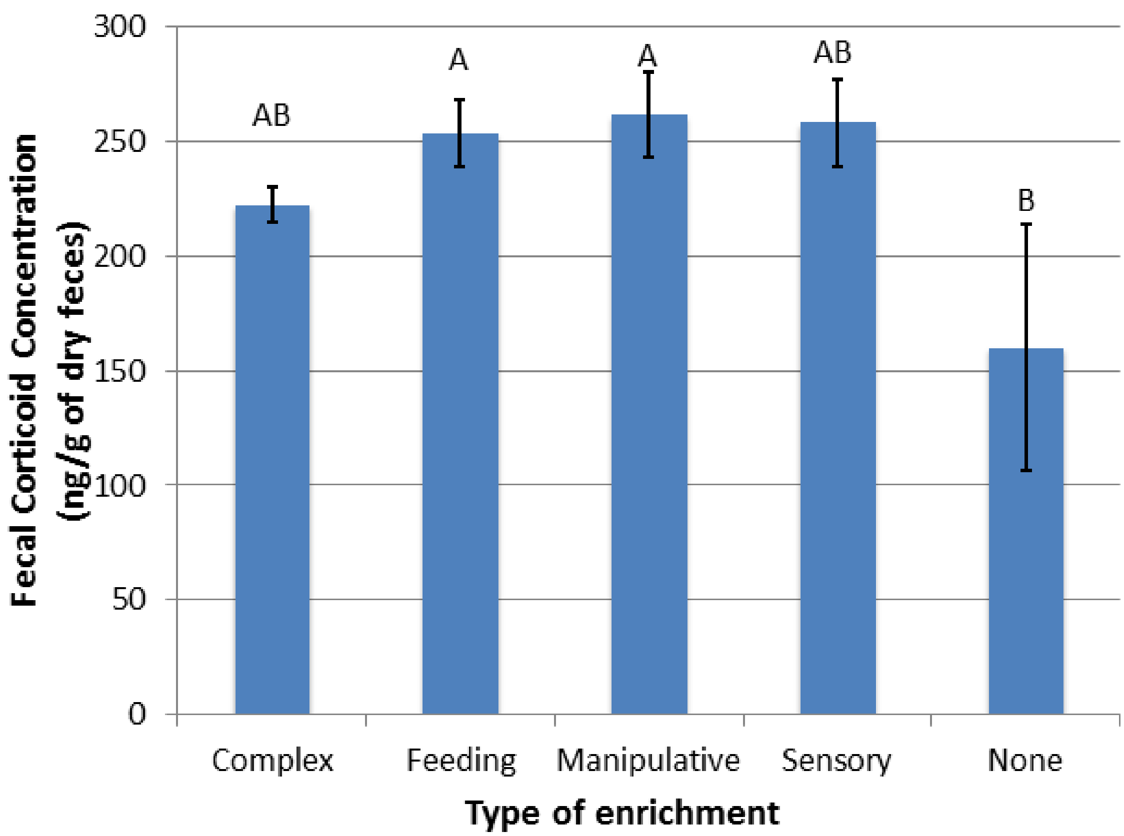

Figure 4.6 Fecal cortisol levels (mean $\pm$SE) of an African lion (L2) in response to different types of enrichment.

Chapter 5: Enrichment Manipulation Experiment

5.1 Overview

The experiment was conducted at Plumpton Park Zoo (PPZ) in Rising Sun, Maryland. Subjects were a Siberian Tiger (T1, 16 yrs) and a cougar (C1, 8 yrs) (Table 5.1). Both cats were females and lived in individual outdoor enclosures approximately 210ft^2 for C1 and 900ft^2 for T3 (Figure 5.1). The enclosures were approximately 80% natural substrate and bordered by chain-linked fencing. Flooring was mostly packed dirt with a small concrete floor in C1's enclosure and a wooden platform in T3's enclosure. The enclosures each had two compartments, separated by a bisecting fence, that were left open continuously throughout the day. If animal care staff required access to one of the compartments (e.g., for feeding and cleaning), the cat could be isolated on the other side allowing the staff safe access to the unoccupied side. C1's enclosure (Figure 5.2a) had a small concrete cave and T3's enclosure (Figure 5.2b) had a small concrete pool.

Subject	Species	Age	Sex
T3	*Panthera tigris altaica*	16	F
C1	*Puma concolor*	8	F

Table 5.1 Subjects at the Plumpton Park Zoo, Rising Sun, MD.

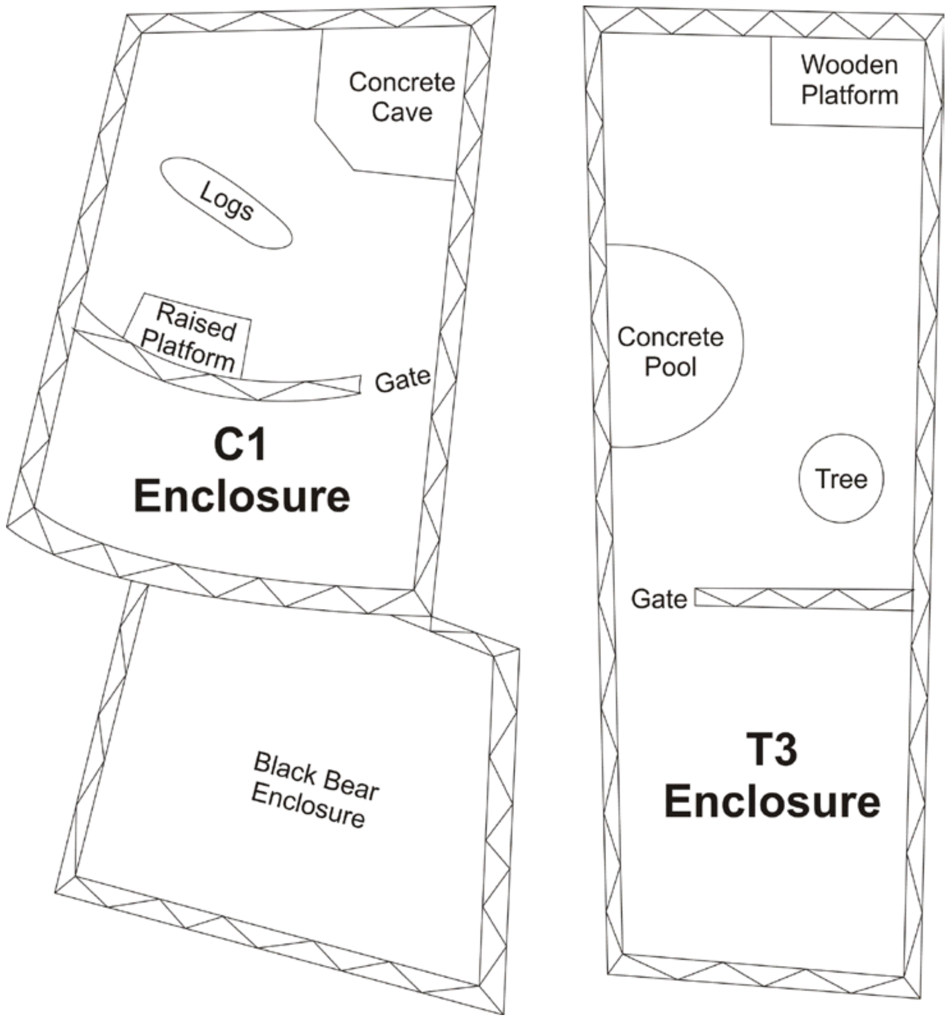

Figure 5.1 Blueprint of the cougar (C1) and tiger (T3) enclosures at PPZ.

Figure 5.2 (a) The cougar enclosure (left) and **(b)** the tiger enclosure (right) at PPZ.

5.2 Methods

5.2.1 Experimental Enrichment Schedule

We created a 9-week enrichment schedule that varied the types and frequency of enrichment. The same schedule of enrichment was used for both subjects. Each program was one week in duration and consisted of either a baseline enrichment program (PPZ's normal enrichment schedule, referred to hereafter as program A), a reduced enrichment schedule (program B), or a novel enrichment schedule modeled on the most frequent enrichment strategies used at the National Zoo (program C). The nine weeks, from July 31st to October 1st of 2011, followed the pattern of A-B-C-B-A-B-C-B-A (see Appendix C for full schedule).

All enrichment programs used feeding, scent, and manipulative enrichments. Program A consisted of seven consecutive days of enrichment using the seven most commonly used enrichment items at PPZ (scatter feeding, hanging paper, phone book with a scent, bloodsicles, snake sheds, catnip in a paper bag, and a novel food in a cardboard box). The reduced enrichment schedule, program B, consisted of the three most frequently used enrichment items from PPZ (scatter feeding, hanging paper, and catnip in a paper bag) interspersed with four days of no enrichment. These were implemented on Monday, Wednesday, and Friday, with no enrichment on remaining days. Program C consisted of seven consecutive days of enrichment using the six most frequent enrichment items used at National Zoo during the summer of 2010 (pepper, fish cubes, bone, rabbit with scent, cardboard, and boomer ball). Each form of enrichment was introduced on a different day, with bone given twice. The complete 9-week schedule was developed before the start of Phase 3 and provided to the animal care staff at PPZ.

The bones, rabbits, and boomer ball were purchased and provided by us. All other items of enrichment from all the programs came from PPZ's own stock. See Appendix D for detailed descriptions of the specific enrichment methods.

5.2.2 Implementation of Enrichment

IACUC approval was obtained prior to enrichment manipulation at PPZ (see Appendix E). Following the schedule above, animal care staff at PPZ introduced the enrichment into the enclosures before morning feeding (approximately 9-12 a.m.) and removed any remnants of the enrichment before evening feeding (approximately 12-4 p.m.). A member of the animal care staff recorded *ad libitum* observations of the animals' reactions to the enrichment. Animal care staff collected feces from the enclosure once per day. Fecal samples were placed in plastic baggies labeled with the animal's identity and date, then stored frozen (-4°C) for later analysis.

Over the nine week period, 43 fecal samples were collected for C1 and 48 fecal samples were collected for T3. Samples were lyophilized and crushed at NZP, then transported to the SBCI Endocrine Research Lab for hormone extraction and EIA as described in Phase 2. Tiger samples were assayed at a dilution of 1:20, while cougar samples were assayed at a dilution of 1:100.

The animal care staff at PPZ wrote brief behavioral observations on the animals' reactions to the enrichment. These behavioral observations were compared to the enrichment schedule we provided PPZ to verify the type of enrichment provided and identify any discrepancies. The only discrepancy between the predetermined schedule and the implementation occurred on 28 August 2011. On this date no enrichment was provided when scatter feeding should have been provided because of zoo clean-up activities necessitated by Hurricane Irene.

After receiving the endocrine values from SBCI, these values were matched up with the enrichment records. Because the endocrine data were not normally distributed, they were log-transformed allowing for statistical analysis. Data were analyzed with ANOVA, with Subject and Treatment as main effects and a Subject*Treatment interaction term.

5.3 Results

The overall model was a good fit for the log-transformed data ($F = 58.6335$, df = 5, 83, $p < 0.0001$). There were significant differences in mean cortisol levels between the two subjects and between different treatments (Table 5.2). The Subject*Treatment interaction was not significant, indicating that both subjects responded similarly to the different enrichment programs.

Source	D.F.	Sum of Squares	F Ratio	Prob > F
Subject	1	9.2121777	264.3396	<.0001*
Treatment	2	0.2171342	3.1153	0.0496*
Subject*Treatment	2	0.0435445	0.6247	0.5379
Error	83	2.892532	0.03485	

Table 5.2 Results of Analysis of Variance on the effects of Subject and enrichment program (Treatment) on fecal cortisol in a female Siberian tiger (T3) and female cougar (C1) maintained at the Plumpton Park Zoo.

C1 showed significantly higher mean cortisol levels than T3 (Figure 5.3, Tukey's HSD, $x = 3.056$, $p < 0.05$). C1 and T3 responded similarly to the different enrichment schedules. Mean cortisol levels were significantly higher for the novel enrichment program compared to the reduced enrichment schedule (Figure 5.4, Tukey's HSD, $p < 0.05$). Mean cortisol levels for the baseline enrichment program were intermediate and did not differ significantly from either the novel enrichment program or the reduced enrichment program.

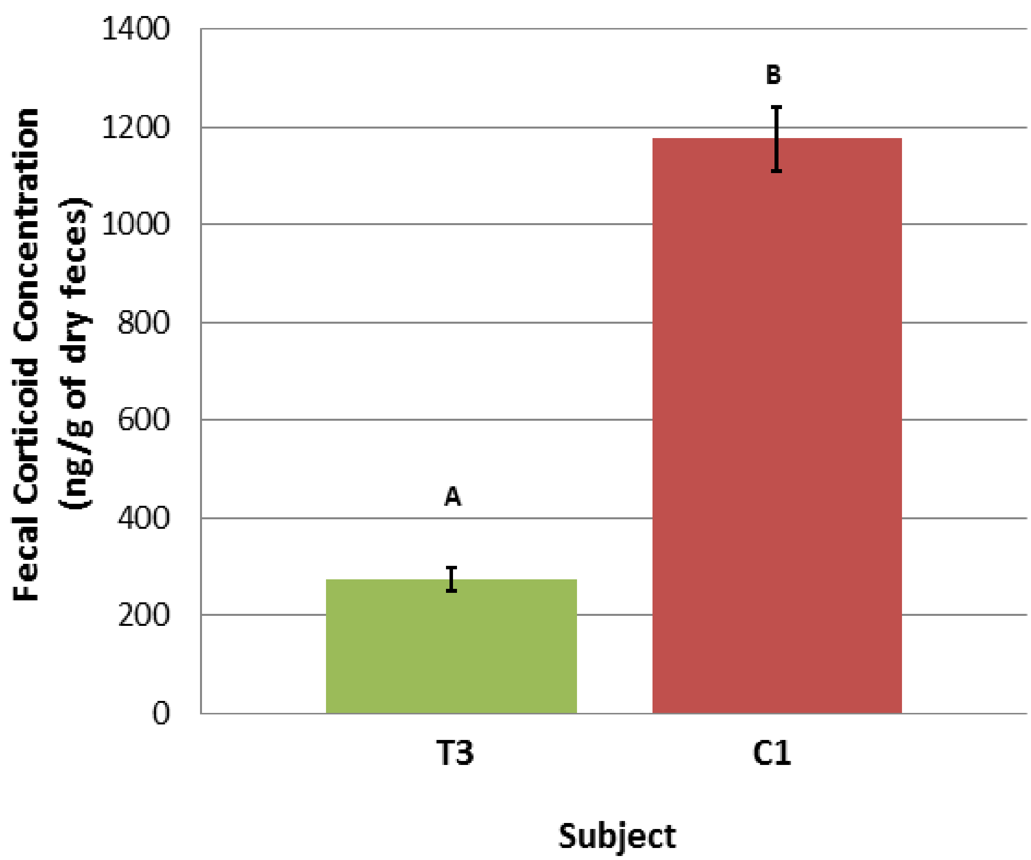

Figure 5.3 Mean overall fecal cortisol levels (+ SE) for a female Siberian tiger (T3) and female cougar (C1) maintained at the Plumpton Park Zoo. Bars with different letters are significantly different from each other.

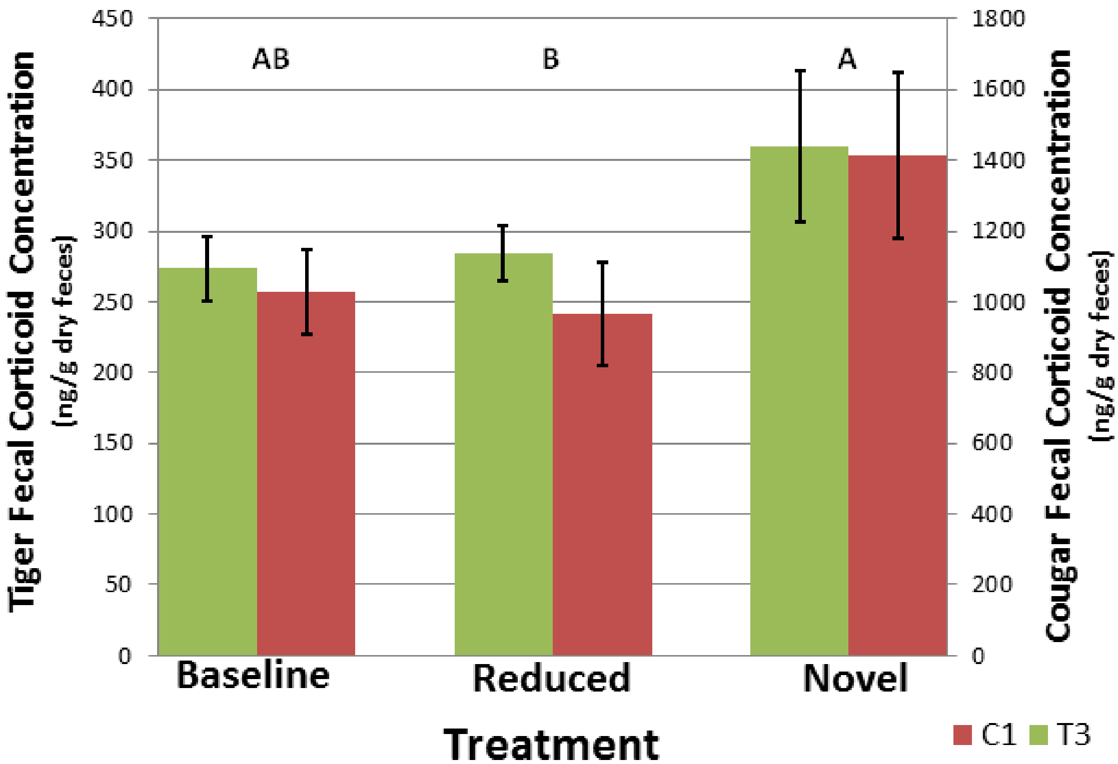

Figure 5.4 Fecal cortisol levels (+SE) for a female Siberian tiger (T3) and female cougar (C1) subject to three different schedules of enrichment at Plumpton Park Zoo. Treatments with different letters are significantly different from each other.

Chapter 6: Discussion

6.1 Factors Affecting Use of Enrichment

The 2011 National Enrichment Practices Survey listed 18 different options for enrichment, and many of these were used by most zoos that responded to the survey. Our results differ somewhat from those of Hoy et al. (2010), who used questions similar to those in our survey, but focused on Australian zoos. In Hoy et al.'s (2010) study, only 67.6% of respondents reported using olfactory enrichment in a given one-week period, while in our study 100% of respondents reported using perfumes and 94% used spices, which are both types of olfactory enrichment. This difference may stem from the more general nature of Hoy et al.'s (2010) survey, which focused on all mammalian species rather than just felids. Hoy et al. (2010) found that feeding enrichment and tactile enrichment were the most important categories of enrichment, with feeding enrichment being used most frequently of all the categories. Our findings were similar, in that feeding enrichments such as fresh meat, produce, ice, frozen blood, and whole animals and animal parts were used by a large majority of responding zoos. The high frequency of use of feeding and tactile enrichment in both American and Australian zoos suggests that these are important enrichment strategies for a diversity of institutions.

AZA-accredited zoos reported implementing enrichment with greater frequency than non-AZA accredited zoos. This finding may reflect the differing emphasis placed on environmental enrichment by the various accrediting organizations. AZA accreditation standards are quite rigorous, requiring that member zoos must "provide an appropriate enriching environment" and stipulating a structure for enrichment oversight (AZA 2011). Other governing bodies, such as ZAA, do not mention standards for the use of enrichment in their accreditation guidelines. While there is no specific AZA requirement for the frequency or types of enrichment used, it is likely that the higher frequency of enrichment at AZA-accredited zoos is due to the strong emphasis that the AZA places on enrichment, including their requirement to have a formal enrichment plan (AZA 2011). Because the practice of enrichment is held in high esteem by the AZA and its member zoos, it is not surprising that these zoos use enrichment more often. It is worth noting that although non-AZA accredited zoos used enrichment less frequently than AZA accredited zoos, all zoos responding to the survey indicated using enrichment to some extent. In the enrichment manipulation experiment, we found similar stress hormone levels when familiar enrichment items were given daily as compared to three times per week, indicating that animals on a reduced enrichment schedule do not exhibit signs of increased stress. This suggests that institutions have some leeway in designing and implementing enrichment programs.

The frequency of enrichment was similar in zoos of different sizes and with different types of ownership. When designing the survey, we expected that these factors would influence enrichment use because smaller zoos might lack the funding to buy enrichment supplies and the staff to implement the enrichment (Fuchs and Ray 2008). Similarly, we suspected that private zoos might have more money, personnel, and resources to devote to implementing enrichment, based on a potentially larger base of financial support. However, neither of these predictions was supported by our survey results. While we are unable to say with certainty why zoos of differing size and ownership are more similar than dissimilar in implementing enrichment, it seems that there is an increasing trend towards the creation of formal enrichment programs for smaller zoos. In Fuchs and Ray's (2008) study, a self-proclaimed "small zoo" created a formal enrichment program that greatly increased enrichment use at that zoo. The authors sought to promote the idea that enrichment programs can be easily implemented regardless of zoo size. However, it is also possible that the recent economic downturn has affected all zoos, decreasing the availability of funds and resources, which in turn has limited enrichment for all institutions and closed any previously existing gap between large and small zoos or public and private zoos.

The 2011 National Enrichment Practices Survey revealed that most zoos felt limited in their use of enrichment. Funding and time were identified as the most limiting factors across all zoo types. Similarly, Hoy et al.'s (2010) survey indicated that time was the most limiting factor for Australian zoos. 70.8% of their respondents reported that the time taken to complete other tasks was often a factor that limited use of enrichment, and only 3.4% stated that time was never a factor. While the percent of zoos responding that time was a limiting factor in our survey (49%) was less than in Hoy et al.'s survey, time was still reported as one of the most important factors limiting enrichment use. In both surveys, about half of respondents reported that human resources were a limiting factor.

It is important to note that Hoy et al.'s (2010) study asked about enrichment practices for all captive mammals, whereas our survey asked about enrichment for lions and tigers specifically. This could account for many of the differences between the two studies, because enrichment strategies differ between species and even individual animals within a species (Swaisgood and Shepherdson 2005). Hoy et al. (2010) also focused on a different part of the world, where husbandry standards may be different.

6.2 Limitations and Future Directions of the Survey

While the 2011 National Enrichment Practices Survey was able to provide useful information on enrichment practices for lions and tigers across the United States, it was not without limitations. First, we chose to disseminate the survey via email, which posed some significant challenges. In several instances, we encountered email addresses that were incorrect or outdated, or we were unable to find an email address for a person associated with felid care at a given facility. While sending a paper survey may have given us a larger potential pool of respondents, it would have made it more difficult to collect and analyze the data because data would have to be entered manually. It also would have made it harder to keep responses anonymous, because each survey would be traceable to a geographic location by its return address or postmark. Thus, an online survey is still a good option for future surveys of this type. However, relying exclusively on electronic communication may have contributed to our small sample size, particularly with regard to non-AZA accredited zoos, for which it was harder to find appropriate email addresses. Because more than 90% of zoos are non-AZA accredited and we received responses from just 11 such zoos, our sample is not generally representative of American zoos. To get a large, truly representative sample, increased communication with and buy-in from non-AZA-accredited zoos would be required. This will be hard to achieve, however, because non-accredited zoos are difficult to locate and are not part of an established community.

Second, the questions in the survey also could have been improved to focus more precisely on the issues we sought to investigate. We asked many questions about what zoos do, but not enough about why they do them, which made it more difficult to interpret our findings. For example, we asked, “Do you feel your use of enrichment is more limited than you would like?” but did not ask what would constitute the ideal use of enrichment. We also asked what types of enrichment zoos use, but not which ones they would adopt if there were no limitations. There were also some terms that were used in the survey that were perhaps difficult for respondents to interpret, and therefore might have affected our results. We did not, for example, distinguish between “time” and “manpower” in the limiting factors section of the survey, so zoos may have perceived the two as very similar, sometimes selecting one option where both may have been applicable. Focus groups or interviews would be necessary to interpret respondents’ answer choices. To gain a fuller understanding of the factors influencing zoos’ enrichment use, a future study should include more opportunities for respondents to elaborate on their responses and questions probing why they selected particular answers.

Third, our survey employed several free response questions. While these provided interesting information concerning zoo practices, they proved to be difficult to analyze. Because we did not design the survey with the objective of quantifying these unique responses, we were only able to look at each response individually. Using this method, we were only able to identify some of the most common words and phrases to characterize enclosures. Even then, this perception was limited due to the open-ended nature of the questions. While there are established methods to derive meaning from free response questions, it is difficult to do so without an *a priori* hypothesis and if the survey has not been specifically designed with this in mind. Administrators of future surveys should determine if and how they want to use free response questions. Very often, open-ended questions are used extensively in the first iteration of a survey to get a feel for the scope of potential responses. A follow-up survey can then be designed that incorporates the most frequent responses into a more quantifiable format, which can then be used for the final version of the survey. This survey serves as a starting point for developing a more detailed survey.

Based on our findings, we recommend that a future study should examine the differences between AZA accredited zoos and non-AZA accredited zoos in greater depth, because that is where we found the greatest differences between zoos in use of enrichment. By asking the same questions, the validity of our findings would be strengthened, and then by asking follow-up questions it would be possible to determine the precise reasons for the differences between AZA accredited and non-AZA accredited zoos.

6.3 Individual Differences in Fecal Cortisol Levels

There were significant individual differences in fecal cortisol in both Phase 2 and Phase 3. NZP subject T1 exhibited significantly higher mean cortisol production than all other NZP subjects, even though each was monitored over the same period of time and experienced similar enrichment schedules. This is consistent with descriptions found in the literature of personality and temperament affecting responses to stress. Boissy et al. (2007) describe "long-term emotional states" affecting an animal's response to stressors, and Wielebnowski (1999) presents many cases of different dispositions leading to different coping mechanisms and behavioral responses to stress. Reeder and Kramer (2005) also assert that behavioral differences can lead to varying hormonal responses to stress.

Several factors may have contributed to T1's increased cortisol levels. T1 was also the oldest subject at 19 years, and Reeder and Kramer (2005) state that responses to stressors often change with age; at more than triple the age of the other subjects, T1 may have developed entirely different endocrine responses to stress. Finally, T1 is female, which may contribute to her different response. Wielebnowski (1999) suggested that because female cheetahs raise and protect their cubs alone, fearfulness is of more importance than to male cheetahs. Female tigers also raise cubs on their own, which may explain why T1 exhibited higher cortisol levels than the other subjects while L1 and L2 showed no statistically significant differences in mean cortisol production (as lions raise their young with an entire pride). While the literature indicates that there are differences in cortisol response between species (Skibiel et al. 2007; Clubb and Mason 2007), because of the small sample size it is impossible to tell whether lions and tigers display different cortisol responses, or whether individual factors were responsible for the higher cortisol levels observed in T1.

Additionally, we found one of the NZP felids (L2) showed changes in cortisol levels in response to different types of enrichment, whereas the other study subjects did not. This again speaks to the highly individual nature of response to different enrichment types and programs. This female lion was also experiencing her first pregnancy, which may have heightened her sensitivity to the presence of enrichment.

Individual differences in cortisol levels were also identified at PPZ. The cougar (C1) had significantly higher cortisol levels than other subjects. However, the cortisol levels of C1 fall within previously recorded cortisol levels for cougars (Bonier et al. 2004).

Skibiel et al. (2007) and Van Metter et al. (2008) both demonstrated that different species can react differently to different types of enrichment. The former study noted that lions exhibited the smallest change in quantity of active behaviors compared to tigers and cougars, while in contrast, the latter found that lions exhibited significantly higher levels of active behaviors in response to enrichment application. Despite the discrepancies between these two studies, they each demonstrate variation between species. Clubb and Mason (2007) claim that different species exhibit different changes in hormone secretion in response to even slight environmental changes. These findings indicate that enrichment programs may need to be tailored to individual animals, rather than generalized for a given species.

It is important to note that differences in fecal cortisol levels do not necessarily reflect differences in serum cortisol, since two animals with identical serum cortisol levels could produce different fecal cortisol levels due to differences in diet, gut retention time, or excretion (Hodges et al. 2010; Terio et al. 1999). We expect these factors to have minimal impact on our study, however, because the subjects received identical diets.

6.4 Effects of Enrichment Methods on Fecal Cortisol Levels

The individual types of enrichment used in this study can be grouped into four categories: feeding, sensory, manipulative, and complex. For at least one subject at NZP, manipulative enrichment elicited the highest cortisol responses. No previous studies have compared the effectiveness of one type of enrichment to another. Hoy et al. (2010) asserted that keepers felt feeding enrichment was the most important type of enrichment for animals in zoos, although the author presents no behavioral or hormonal data to support that assertion.

The novel enrichment treatment at PPZ produced significantly higher mean cortisol levels in both subjects compared to the reduced treatment. Higher mean cortisol levels signify a higher level of stress in the animal (Wielebnowski 2009), indicating that the novel enrichment treatment produced a higher level of stress for both felids. However, cortisol levels alone cannot determine whether this higher stress level is from eustress or distress (Wielebnowski 2009). Eustress can result from increased activity levels and excitement, while distress can result from fearful situations or boredom (Ladewig 2000). The novel enrichment items could have caused excitement and higher activity levels (indicating eustress) or increased fearfulness (indicating distress). Behavioral observations would be necessary to differentiate between these alternatives.

Both the reduced and the baseline enrichment treatments were based on the most frequent enrichment items previously used at PPZ, whereas the novel enrichment treatment introduced different enrichment items. Because the reduced and baseline enrichment items were previously used, habituation may have been a factor. Mellen and Shepherdson (1997) found that felids eventually habituated behaviorally to enrichment items that they encounter repeatedly, while Van Metter et al. (2008) found that, over a trial period, tigers and lions did not behaviorally habituate to enrichment items such as blood balls, zebra dung, and cardboard boxes. Because both the reduced and baseline enrichment treatments contained items previously used at PPZ, the items were no longer novel. The subjects may have habituated to these enrichment items, resulting in decreased activity levels, decreased levels of eustress, and lower mean cortisol levels. Items in the novel enrichment treatment may have induced higher activity levels because of their novelty. The increased levels of cortisol and corresponding stress response may have elicited the benefits associated with hormesis as well. Without behavioral observations, determining whether the higher mean cortisol levels are due to eustress, distress, habituation, or some combination is not possible. The time course of this study was too short to determine if immune function was improved due to eustress exposure, and none of the animals was ill during our study period. The effect of novel enrichment on immune system function would be a fruitful avenue of research for a future study.

6.5 Limitations of Enrichment Study

Given our small sample size, it is difficult to make generalizations regarding the efficacy of different types of enrichment. However, if sample size could be expanded by including several additional institutions, a more extensive examination of the effects of different types of enrichment could be made. If both fecal samples and behavioral observations were obtained, it would provide a more comprehensive evaluation of the effect of enrichment.

Our study did not identify any seasonal or daily trends in cortisol response. Therefore, we were able to eliminate seasonal and daily trends as potential confounding variables that could have threatened the validity of our results. However, had we been able to perform this study over a longer period of time, we might have seen seasonal effects. Seasonal changes were positively correlated with fecal glucocorticoid metabolite secretion in captive and wild Canada lynx (*Lynx Canadensis*) (Fanson et al. 2012). In males, fecal glucocorticoid metabolite secretions increased during mating season in early spring and decreased during the summer. By contrast, in females, fecal glucocorticoid metabolite secretions peaked after the breeding season and decreased during the winter or early spring. Factors that play a role in variation of glucocorticoid levels include changes in food availability/energy regulation and reproduction (Boonstra 2004; Romero 2002; St. Aubin et al. 1996; Touma and Palme 2005).

Though our study raises some concerns about the generalizability of our results due to a small sample size, the validity of the results is supported by the similarity in endocrine responses by both PPZ subjects. Each of the animals displayed increased cortisol levels when presented with novel enrichments. Since the two subjects were of different felid species, this suggests that the response pattern may be general. Further work with a larger number of species and individuals is required to confirm the generalizability of the findings.

Another limitation of our study was a low frequency of fecal sampling. Samples were collected from NZP an average of 1.5 times per week, and from PPZ an average of 4.5 times per week. Factors that prevented daily collection of samples include the keepers' inability to find all fecal samples within the enclosure, as well as the tendency of individual animals to defecate less frequently than once per day. This meant that we did not have a fecal sample to correlate to each type of enrichment for each animal.

There may also have been additional limitations to our ability to measure fecal cortisol accurately. It is possible that some of the fecal samples were misattributed to other animals sharing the same enclosure, or that they were contaminated with another individual's urine during territorial scent marking. Other factors that may have decreased sample reliability include freshness of the sample and possible environmental contamination of the sample (Wielebnowski and Watters 2007).

A final limitation of the study was its reliance on endocrine data alone. Fecal corticoid measurements are most valuable when analyzed in the context of additional data, such as behavioral observations. Given the distance of PPZ from our main campus, it was not feasible for us to collect supplemental behavioral data. Such observations would be required to identify whether the endocrine responses of the animals are a result of distress or eustress in each enrichment scheme.

Chapter 7: Conclusion

The harmful effects of captivity on animals can manifest in decreased fecundity, abnormal behaviors and generally reduced well-being. Captive felids are especially susceptible to these adverse effects and the zoo community has been working towards improving the captive environment for these species, often through the application of environmental enrichment. Our team's goal was to research common enrichment practices used nationwide, understand the effects of a predetermined enrichment schedule on the cortisol levels of captive felids, and then build on this background knowledge to design a novel enrichment schedule that would enable us to investigate the effects of enrichment on cortisol levels. Our experimental enrichment schedule demonstrated that the introduction of novel enrichment resulted in significantly higher cortisol levels for our subjects when compared to weeks with reduced, familiar enrichment. These increased cortisol levels could be indicative of increased activity or interaction, rather than boredom or anxiety, but without being paired with behavioral observations, it is difficult to discriminate between these alternatives.

Through the *2011 National Enrichment Practices Survey*, we learned that the majority of zoos, whether they are large or small, public or private, AZA-accredited or not, face similar issues and limitations with regards to environmental enrichment. The large majority of zoos (78% of respondents) had a very modest ($0-$50) monthly budget for enrichment, and donations constituted a portion of the resources used. Limitations faced universally by zoos included funding, manpower, and time. Often, keeper preferences dictated the enrichment used. Thus, the enrichment programs were tailored to suit the needs of the institution as well as the individual animal. As a result, enrichment programs may need to be established based on the unique requirements of the animals and the resources available to zoo staff, rather than being uniform across species or institutions. Although different zoos will have different resources and institute-specific limitations, our data suggest that animals habituate to enrichment programs, so any enrichment plan should contain strategies for allowing animals to experience novelty.

A certain degree of flexibility is also necessary, which became increasingly evident to our team as our research progressed. Our experiment faced the same issues voiced by the respondents in our survey. While some of the enrichment items used by PPZ in the experimental enrichment phase were of their own stock, we did provide Boomer balls, cow femurs and frozen rabbit carcasses. Overall, this corresponded to spending approximately $30 per animal for one week of novel enrichment. However it is important to mention that this estimate excludes the one-time investment of $135 for a Boomer ball, an enrichment item that can be used repeatedly. These estimates are important to consider for zoos wishing to adopt a similar enrichment program.

By working with this group of institutions, we recognized the importance of inter-institution cooperation in the development of research and new ideas. This allowed for a combination of resources, expertise, and advice on the direction of our project. The development of novel enrichment programs may need to be conducted on an animal by animal basis, but the sharing of ideas and data between institutes will further the zoo community's efforts to improve the well-being of their charges. By implementing enrichment programs that encourage natural behaviors in captive animals, especially those that are endangered, the zoo community will be better able to conduct accurate research, educate the public, and strengthen conservation efforts.

Appendices

Appendix A – 2011 National Enrichment Practices Survey

Thank you for participating in our survey! Our goal is to examine the enrichment practices of zoos nationwide with regards to lions and tigers. In order to determine what forms of enrichment we'd like to investigate, we first need to gather more data on what forms of enrichment are currently commonly used in zoos. We would greatly appreciate if you could complete this electronic survey, which should take no more than 10 minutes. Your participation is extremely important for the validity and success of our project. Your responses to this survey are strictly confidential.

Respondents will be kept up to date on the progress of our research, along with receiving feedback on the study results. We plan to publish our research results in our Spring 2012 thesis, and will be happy to send this to you if you indicate your interest by emailing us at teamblaze2012@gmail.com.

* Required

Lions and Tigers

How many lions does your institution have?* __________

How many tigers does your institution have?* __________

Approximately what percentage of the lion exhibit is natural substrate?
Natural substrate being grass, dirt, etc.

	1	2	3	4	5	6	7	8	9	10	
0%	○	○	○	○	○	○	○	○	○	○	100%

Describe the landscape and fixtures of the enclosure for LIONS (if applicable). Please be sure to include the approximate size of the enclosure.
Features such as caves, hiding places, moats, etc.

On average, how many hours of the day is each lion on exhibit? __________

Approximately what percentage of the tiger exhibit is natural substrate?
Natural substrate being grass, dirt, etc.

	1	2	3	4	5	6	7	8	9	10	
0%	○	○	○	○	○	○	○	○	○	○	100%

Describe the landscape and fixtures of the enclosure for TIGERS (if applicable). Please be sure to include the approximate size of the enclosure.
Features such as caves, hiding places, moats, etc.

On average, how many hours of the day is each tiger on exhibit? __________

Lion and Tiger Enrichment Program

Which of the following forms of enrichment do you use for lions and tigers? Select all that apply.

- o Frozen meat (e.g., meatsicles, marrowsicles, frozen beef chunks)
- o Fresh meat (e.g., meatballs)
- o Whole animals and part (e.g., bloodsicles, bloodballs)
- o Frozen fish (e.g., fish cubes)
- o Ice
- o Animal scenes
- o Animal feces
- o Spices
- o Catnip
- o Perfume
- o Cardboard, boxes
- o Boomer ball

- o Log
- o Burlap bag
- o Papier-mâché object/piñata
- o Mirrors
- o Large produce/melons

If you use SCENTS, please provide further details on their implementation below.
Which scents you use, how you apply them, etc.

If you wish to elaborate on the implementation of any other form of enrichment (specifics, effectiveness, etc.) feel free to do so in the space provided:

How often do you implement some form of enrichment?

- o Daily
- o Multiple times per week
- o At least once a week
- o Sporadically

Do you feel your use of enrichment is more limited than you would like?

- o Yes
- o No

If so, which factor(s) do you find most limiting? Mark all that apply.

- o Funding
- o Manpower
- o Suppliers

- Time
- Approval process
- Issues with individual animals
- Other:

Approximately how much do you spend on enrichment per month?

- $0-50
- $50-100
- $100+

Background Information

In which state are you located?* _________

Which of the following organization is your institution accredited by, if any?*
Select all that apply.

- Association of Zoos and Aquariums (AZA)
- Zoological Association of America (ZAA)
- American Sanctuary Association (ASA)
- Other:

Approximately how many species do you have?*
Including birds and invertebrates.

- 0-100
- 100-500
- 500-1000
- 1000+
- I don’t know

About how many zoo employees are engaged in animal care?*

- 0-15
- 16-30
- 31-45
- 46-60
- 60+

Approximately how many zoo employees are engaged in care of large cats?*

____

During peak season, approximately how many people visit the zoo on a daily basis?*

- 0-1000
- 1000-2000
- 2000-3000
- 3000-4000
- 4000+

Is your institution privately owned?*

- Yes
- No

Appendix B – Enrichment Logs from NZP 2010

Enrichment logs for L1, L2, L3, T1, and T2 from the Smithsonian National Zoological Park, Washington, DC for the summer of 2010.

Date	L1, L2, L3 Enrichment	T1 Enrichment	T2 Enrichment
1-Jun	Herbivore poop	Bloodsicle in box	Bloodsicle in box
2-Jun	Keg	Scents	Boomer ball
3-Jun	Bones	Ox tail	Bone
4-Jun	Scents	Melon with catnip	Log with catnip
5-Jun	Fish cubes	None	None
6-Jun	Bones	Ox tail	Bone
7-Jun	Rabbit w/ scent	Bag with scent	Bag with scent
8-Jun	Cardboard	Boomer with scent	Bloodsicle
9-Jun	Boomer ball	None	None
10-Jun	Bones	Ox tail in scented box	Bone in box
11-Jun	Anteater logs	Keg	Scent
12-Jun	Bloodsicles	Boomer with scent	Keg
13-Jun	Bones	Marrowsicle	Bone
14-Jun	Rabbit w/ scent	Frozen cake	Frozen cake
15-Jun	Burlap sacks	None	None
16-Jun	Herbivore poop	Scents	Scents
17-Jun	Bones	Oxtail	Bone
18-Jun	Boomer ball	None	None
19-Jun	Fish cubes	Marrowsicle	Marrowsicle
20-Jun	Bones	Ox tail	Bone
21-Jun	Rabbit w/ scent	Rabbit	Rabbit
22-Jun	Cardboard	Frozen beef chunks	Frozen beef chunks
23-Jun	Keg	Frozen beef chunks	Frozen beef chunks
24-Jun	Bones	Ox tail	Bone
25-Jun	Scents OR Papier Mache Animal	None	None
26-Jun	Bloodsicles	Bloodsicle	Bloodsicle
27-Jun	Bones	None	None
28-Jun	Rabbit	rabbit	rabbit
29-Jun	Anteater logs	None	None
30-Jun	Cardboard	Burlap with scent	boxes
1-Jul	Bones	None	None
2-Jul	Burlap sacks	mirrors	boomer on a spring
3-Jul	Fish cubes	None	None
4-Jul	Bones and keg	ox tail	bone

5-Jul	None recorded OR Rabbit	rabbit	rabbit
6-Jul	Herbivore poop	bloodsicle	scented icicle
7-Jul	Bloodsicles WITH/OUT Keg	meatsicle	meatsicle
8-Jul	Bones	ox tail	bone
9-Jul	Scents	boomer with scent	bag with scent
10-Jul	Keg	meatsicle	meatsicle
11-Jul	Bones and cardboard OR Bones	rabbit	rabbit
12-Jul	Rabbit (bones for females only)	None	None
13-Jul	Cardboard	None	None
14-Jul	None recorded OR Boomer Ball	bloodsicle	meatballs hidden in yard
15-Jul	Bones	ox tail	bone
16-Jul	Anteater logs	scents	scents
17-Jul	Fish cubes	bloodsicle	keg
18-Jul	Bones	ox tail	bone
19-Jul	Rabbit	rabbit	rabbit
20-Jul	Burlap sacks	bloodsicle	None
21-Jul	None recorded OR Keg	None	blood ball
22-Jul	Bones WITH/OUT Keg	None	None
23-Jul	Scents	boomer	keg
24-Jul	Bloodsicles WITH/OUT Fish cubes	keg	meatsicle
25-Jul	Bones WITH/OUT Scented ice blocks	bone	bone
26-Jul	None recorded OR Rabbit	rabbit	rabbit
27-Jul	Boomer ball OR Cardboard	bloodsicle	bloodsicle
28-Jul	Boomer ball	None	None
29-Jul	Bones	None	None
30-Jul	None recorded OR Papier Mache Animal	None	None
31-Jul	Fish cubes	boomer	None
1-Aug	Bones	-	-
2-Aug	Rabbit	-	-
3-Aug	Herbivore poop	-	-
4-Aug	Keg	-	-
5-Aug	Bones	-	-
6-Aug	Burlap sacks	-	-
7-Aug	Bloodsicles	-	-
8-Aug	Bones	-	-

9-Aug	Rabbit	-	-
10-Aug	Cardboard	-	-
11-Aug	Boomer ball	-	-
12-Aug	Bones	-	-
13-Aug	-	-	-
14-Aug	Fish cubes	-	-
15-Aug	Bones	-	-
16-Aug	Rabbit	-	-
17-Aug	Burlap sacks	-	-
18-Aug	-	-	-
19-Aug	Bones	-	-
20-Aug	Scents	-	-
21-Aug	Bloodsicles	-	-
22-Aug	Bones	-	-
23-Aug	Rabbit	-	-
24-Aug	Cardboard	-	-
25-Aug	Keg	-	-
26-Aug	Bones	-	-
27-Aug	Anteater logs	-	-
28-Aug	-	-	-
29-Aug	Bones	-	-
30-Aug	Rabbit	-	-
31-Aug	Herbivore poop	-	-
1-Sep	Fire hose	Ball with scent	Scent
2-Sep	Bones	Oxtail	Bones
3-Sep	Burlap sacks	None	None
4-Sep	Bloodsicles and keg for Luke	Chunksicle	Bloodsicle and Keg
5-Sep	Bones	Bone	Bones
6-Sep	Rabbit (Naba inside)	Rabbit	Rabbit
7-Sep	Cardboard	None	None
8-Sep	Boomer ball	None	None
9-Sep	Bones (Fire hose?)	Oxtail	Bones
10-Sep	Scents	Scent	Scent
11-Sep	Fish cubes	Fire hose	Keg
12-Sep	Bones	Oxtail	Bones
13-Sep	Rabbit (Naba inside)	Rabbit	Rabbit
14-Sep	Luke - bloodsicles; ??	Small boomer ball	Boomer Ball???
15-Sep	Keg	None	Boomer Ball???
16-Sep	Bones (Luke only)	None	None
17-Sep	??? - bones	Cardboard	Cardboard

18-Sep	Bloodsicles	Bloodsicle	Keg
19-Sep	Bones	Oxtail/Fiesta Musical	Bones
20-Sep	Keg	Rabbit	Bag
21-Sep	Herbivore poop	None	None
22-Sep	Fire hose	None	None
23-Sep	Bones	Oxtail	Bones and box
24-Sep	Burlap sacks	None	None
25-Sep	Fish cubes	None	None
26-Sep	Bones (Luke)	None	None
27-Sep	Scents	Rabbit	Rabbit
28-Sep	Cardboard	None	Scatter feed
29-Sep	Boomer ball	None	None
30-Sep	Bones	None	Bone
1-Oct	Scents	None	None
2-Oct	Bloodsicles	None	None
3-Oct	Bones	Oxtail	Bone
4-Oct	Rabbit	Rabbit	Beef chunk pop and Rabbit
5-Oct	Cardboard	Chunksicle	None
6-Oct	Fire hose	None	None
7-Oct	Bones	Oxtail/Mesh access with Gunther	Bone/mesh access with Soy
8-Oct	Burlap sacks	Rosemary	Rosemary
9-Oct	Fish cubes	None	None
10-Oct	Bones	Oxtail	Bone
11-Oct	Rabbit	Rabbit	Rabbit (inside)
12-Oct	Herbivore poop	None	Training with feed stick
13-Oct	Keg	None	Trained in front of crowd
14-Oct	Bones	Oxtail	Bone
15-Oct	Scents	None	None
16-Oct	Bloodsicles	Burlap with scent	Keg
17-Oct	Bones	Bone	Bone
18-Oct	Rabbit	Rabbit	Rabbit, cardboard inside
19-Oct	Cardboard	Oxtail; cardboard with spearmint	None
20-Oct	Boomer ball	None	Meatballs
21-Oct	Bones	None	Bone in tree
22-Oct	Scents	Anteater Log	Logs, pumpkin with ox tail
23-Oct	Fish cubes	None	None

24-Oct	Bones	Pumpkin in moat	Pumpkin in moat and Bone
25-Oct	Rabbit	Rabbit	Pumpkin in tree and Rabbit
26-Oct	Anteater logs	Pumpkin in den	Burlap sack
27-Oct	Keg	None	None
28-Oct	Bones	None	None
29-Oct	Burlap sacks	Yard switch	Yard switch
30-Oct	Bloodsicles	Lion's burlap	Lion's burlap
31-Oct	Bones	None	Bone

Appendix C – Enrichment Manipulation Schedule

Enrichment schedule for C1 and T3 at Plumpton Park Zoo, Rising Sun, Maryland from August 1st to October 1st of 2011

	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Week 1: July 31-Aug 6	Scatter Feeding	Hanging Paper	Phone Book + Scent	Bloodsicles	Sheds	Catnip in Paper Bag	Novel Food in Box
Week 2: Aug 7-13	Nothing	Scatter Feeding	Nothing	Hanging Paper	Nothing	Catnip in Paper Bag	Nothing
Week 3: Aug 14-20	Scent: Pepper	Fish Cubes	Bone	Rabbit + Scent	Card-board	Boomer Ball	Bone
Week 4: Aug 21-27	Nothing	Scatter Feeding	Nothing	Hanging Paper	Nothing	Catnip in Paper Bag	Nothing
Week 5: Aug 28-Sep 3	Scatter Feeding*	Hanging Paper	Phone Book + Scent	Bloodsicles	Sheds	Catnip in Paper Bag	Novel Food in Box
Week 6: Sep 4-10	Nothing	Scatter Feeding	Nothing	Hanging Paper	Nothing	Catnip in Paper Bag	Nothing
Week 7: Sep 11-17	Scent: Pepper	Fish Cubes	Bone	Rabbit + Scent	Card-board	Boomer Ball	Bone
Week 8: Sep 18-24	Nothing	Scatter Feeding	Nothing	Hanging Paper	Nothing	Catnip in Paper Bag	Nothing
Week 9: Sept 25-Oct 1	Scatter Feeding	Hanging Paper	Phone Book + Scent	Bloodsicles	Sheds	Catnip in Paper Bag	Novel Food in Box

*No enrichment was provided due to clean-up necessitated by hurricane clean up

Appendix D – Enrichment Item Definitions

Descriptions of various enrichment used at the National Zoological Park and Plumpton Park Zoo

Item	Type of Enrichment	Description/Implementation
Bloodsicle	Feeding	Frozen mixture of water and blood given like a food treat
Bone	Feeding	Bone placed in enclosure for chewing and gnawing
Boomer Ball	Manipulative	Heavy duty plastic ball placed in enclosure for play and manipulation
Burlap Bags	Manipulative	Empty or hay-filled burlap bag placed in enclosure for play and manipulation
Cardboard	Manipulative	Cardboard boxes or pieces placed in enclosure for play and manipulation
Fish Cubes	Feeding	Frozen pieces of fish given in addition to regular diet
Herbivore Poop	Sensory	Herbivore feces placed in enclosure for sensory stimulation
Keg	Manipulative	An empty metal keg or plastic drum placed in enclosure for play and manipulation
Log	Manipulative	Tree log placed in enclosure for play and manipulation
Paper Mache Animal/Object	Manipulative	Large paper mache object placed in enclosure for play and manipulation
Rabbit	Feeding	Frozen rabbit given in addition to regular diet
Scent	Sensory	Different scents (animal scents, perfume, etc.) sprayed on objects within the enclosure
Scatter Feeding	Feeding	Small pieces of meat hidden throughout enclosure in addition to regular diet
Hanging Paper	Manipulative	Pieces of plain paper hung on tree limbs and other overhangs throughout enclosure for play and manipulation
Phone Book + Scent	Manipulative Sensory	Phone book that has been sprayed with a scent placed inside in enclosure for play and sensory stimulation
Sheds	Sensory	Snake skin sheds placed inside enclosure for sensory stimulation
Catnip in Paper Bag	Sensory	Catnip placed inside a paper bag and then placed within the enclosure for play and sensory stimulation
Novel food in Box	Feeding Manipulative	Novel meat that is not a regular part of the diet placed in a box and in enclosure for manipulation and an addition to the regular diet

Appendix E – IACUC Approval Letter

UNIVERSITY OF MARYLAND

DIVISION OF RESEARCH
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July 19, 2011

Dr. Katerina Thompson
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Dr. Thompson,

This letter is to inform you that on **June 5, 2011** the members of the Institutional Animal Care & Use Committee (IACUC) reviewed and approved the protocol for:

Analysis of the Effects of Enrichment on Large Fields

R-11-25

Please note that an approved protocol is valid for three (3) years unless there is a change in the protocol. Thus, this protocol is valid until **June 5, 2014**. Federal laws indicate that protocols must be reviewed yearly. Thus, in order to keep your approved protocol active you **MUST** submit a protocol renewal/update by the first of the month of the anniversary of your approval (June 2012 & June 2013). All work extending beyond the approval date of the protocol must be submitted to the IACUC as a new protocol.

Sincerelly,

W. R Stricklin

W. Ray Stricklin
Asst. Dean, College of Ag. & Natural Resources
Chair, IACUC

CC: Doug Powell, Amanda Underwood

Glossary

Activity Budget: a way of representing animal behavior by recording the duration of defined activities such as feeding, grooming (Graetz 1995)

Biodiversity: the variety of ecosystems, habitats, species, organisms and genes comprising life on Earth (Rands et al. 2010)

Complex Enrichment: any combination of feeding, sensory, or manipulative enrichments (i.e. a cardboard box coated in scent)

Distress: the point when an animal begins to experience negative effects from exposure to stressors (Wielebnowski 2003)

Enrichment: a combination of inanimate and social stimulation to alleviate negative behavior and stimulate natural behavior (Van Praag et al. 2000 and Graetz 1995)

Enzyme Immunoassay: a means of detecting a substance via the use of an antibody bound to an enzyme to emit a visual signal when the antibody binds to the hormone of interest

Eustress: a response to a stressor that elicits an increase in activity levels or excitement, but confers no negative effects (Ladewig 2000)

Fecundity: the ability to produce a viable offspring (McPhee and Carlstead 2010)

Feeding enrichment: "a variety of task oriented puzzle feeders and different methods of presentation encourage animals to think and work for their food, as they would in the wild" (Hogle 2009)

Felids: animal belonging to the Felidae family in the mammalian order Carnivora, more commonly known as the cat family (Feldhamer et al. 2007)

Manipulative Enrichment (aka Tactile Enrichment): "items (such as barrels, balls, or boxes) that can be manipulated in some way via hands, mouth, legs, horns or head simply for investigation and exploratory play" (Hogle 2009)

Natural Substrate: the portion of an animal's enclosure which can be found in the native habitat (i.e. soil, grass, rocks, water, etc.)

Ranging Tendency: the average distance a species travels in a given period of time under a typical, natural setting (Terio et al 2004)

Sensory Enrichment: "techniques such as bubbles, scents, or video recordings that would stimulate…the animals' senses- visual, olfactory, auditory, taste and tactile" (Hogle 2009)

Stereotypies: abnormal repetitive behaviors exhibited by animals, often in response to stress; also known as "stereotypic behaviors" (Pitsko 2003)

Stress: any perturbation from homeostasis (Reeder and Kramer 2005)

Stressor: any stimulus that causes a perturbation from homeostasis

Literature Cited

Altman JD. 1998. Animal activity and visitor learning at the zoo. Anthrozoos 11:12-21.

Anderson, RS. 1995. The Lacey Act: America's premier weapon in the fight against unlawful wildlife trafficking. Public Land Law Review. 16 Pub. L. L.R. 27.

AZA. 2009. *What is Accreditation?.* Association of Zoos & Aquariums. <http://www.aza.org/what-is-accreditation/>

AZA. 2011. *The Accreditation Standards and Related Policies.* Association of Zoos & Aquariums.<http://www.aza.org/uploadedFiles/Accreditation/Accreditation%20Standards.pdf>

AZA. 2012. *The Guide to Certification of Related Facilities.* Association of Zoos & Aquariums. <http://www.aza.org/uploadedFiles/Accreditation/Guide%20to%20Certification.pdf>

Balcom, SA. RE: University of Maryland Undergraduate Research [electronic mail on the Internet]. Message to: Team BLAZE. 2012 Mar. 30 [cited 2012 Mar. 30]. [about 1 paragraph].

Ballantyne R, Packer J, Hughes K, Dierking L. 2007. Conservation learning in wildlife tourism settings: lessons from research in zoos and aquariums. Environmental Education Research 13:367-383.

Bashaw MJ, Bloomsmith MA, Marr MJ, Maple TL. 2003. To hunt or not to hunt? A feeding enrichment experiment with captive large felids. Zoo Biology 22:189-198.

Bashaw MJ, Kelling As, Bloomsmith MA, Maple TL. 2007. Environmental effects on the behavior of zoo-housed lions and tigers with a case study of the effects of a visual barrier on pacing. Journal of Applied Animal Welfare Science 10:95-109.

Bauer H, Van Der Merwe S. 2004. Inventory of free-ranging lions *Panthera leo* in Africa. Oryx 38:26-31.

Boissy A, Manteuffel G, Jensen MB, Moe RO, Spruijt B, Keeling LJ, Winckler C, Forkman B, Dimitrov I, Langbein J, Bakken M, Veissier I, Aubert A. 2007. Assessment of positive emotions in animals to improve their welfare. Physiology and Behavior 92:375-397.

Bonier F, Quigley H, Austad SN. 2004 A technique for non-invasively detecting stress response in cougars. Wildlife Society Bulletin 32(3):711-717.

Boonstra R. 2004. Coping with changing northern environments: the role of the stress axis in birds and mammals.: Integrative & Comparative Biology. p. 95-108.

Chivian E. 2003. *Biodiversity: Its Importance to Human Health.* Cambridge: Harvard Medical School.

Churchman D. 1987. The educational role of zoos: A synthesis of the literature (1928-1987) with annotated bibliography. ERIC Document Reproduction Service No. ED287942

Clark F, King AJ. 2008. A Critical Review of Zoo-based Olfactory Enrichment. In *Chemical Signals in Vertebrates 11.* Ed. JL Hurst, RJ Beynon, S Craig Roberts, and TD Wyatt. New York: Springer.

Clubb R, Mason GJ. 2003. Animal welfare: captivity effects on wide-ranging carnivores. Nature 425:473-474.

Clubb R, Mason GJ. 2007. Natural behavioural biology as a risk factor in carnivore welfare: How analysing species differences could help zoos improve enclosures. Applied Animal Behaviour Science 102(3-4):303-328.

Cohen S, Kessler RC, Underwood Gordon L. 1997. Strategies for measuring stress in studies of psychiatric and physical disorders. In *Measuring Stress; A Guide for Health and Social Scientists.* Ed. S Cohen, RC Kessler, and L Underwood Gordon. New York: Oxford University Press.

Conway WG. 2011. Buying time for wild animals with zoos. Zoo Biology 30:1-8.

Duffy JE. 2009. Why biodiversity is important to the functioning of real-world ecosystems. Frontiers in Ecology and the Environment 7: 437-444.

Ebersole RS. 2001. *The New Zoo*. Audubon Magazine. < http://archive.audubonmagazine.org/features0111/newzoo.html>

Fanson KV, Wielebnowski NC, Shenk TM, Lucas TR. 2012. Comparative patterns of adrenal activity in captive and wild Canada lynx (*Lynx canadensis*). J Comp Physiol B 182:157-165.

Feldhamer GA, Drickamer LC, Vessey SH, Merritt JF, Krajewski C. 2007. *Mammalogy: Adaptation, Diversity, and Ecology*, 3rd Ed. Baltimore: The Johns Hopkins University Press.

Fraser D. 2009. Assessing animal welfare: different philosophies, different scientific approaches. Zoo Biology 28:507-518.

Fuchs MA, Ray HL. 2008. Design of a formal animal enrichment program for a small zoological institution: from realization to adaptive management. American Association of Zoo Keepers National Conference Proceedings. <http://aazk.org/members/files/2008%20proceedings/Fuchs.pdf>

Gillespie TH. 1934. *Is it Cruel? A Study of the Condition of Captive and Performing Animals.* London: Herbert Jenkins.

Gittleman JL, Harvey PH. 1982. Carnivore home-range size, metabolic needs and ecology. Behavioral Ecology and Sociobiology 10:57-63.

Graham LH, Brown JL. 1996. Cortisol metabolism in the domestic cat and implications for non-invasive monitoring of adrenocortical function in endangered felids. Zoo Biology 15(1):71-82.

Grech KS. 2004. Detailed discussion of laws affecting zoos. Animal Legal and Historical Center.

Haas SK, Hayssen V, Krausman PR. 2005. *Panthera leo*. Mammalian Species, No. 762:1-11.

Hancocks D. 2010. The History and Principles of Zoo Exhibition. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Hayward MW, Kerley GIH. 2005. Prey preferences of the lion (*Panther leo).* J. Zool., Lond. 267:309-322.

Hediger H. 1950. *Wild Animals in Captivity: An Outline of the Biology of Zoological Gardens.* London: Butterworths Scientific Publications.

Hediger H. 1955. *Studies of the Psychology and Behaviour of Captive Animals in Zoos and Circuses.* London: Butterworths Scientific Publications.

Hediger H. 1969. *Man and Animal in the Zoo*. New York: Delacorte Press.

Heinsohn R, Packer C. 1995. Complex cooperative strategies in group-territorial African lions. Science 269:1260-1262.

Hill SP, Broom DM. 2009. Measuring zoo animal welfare: theory and practice. Zoo Biology 28:531-544.

Hodges K, Brown K, Heistermann M. 2010. Endocrine Monitoring of Reproduction and Stress. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. D Kleiman, K Thompson, and C Baer. Chicago: The University of Chicago Press.

Hoy JM, Murray PJ, Tribe A. 2010. Thirty years later: enrichment practices for captive mammals. Zoo Biology 29:303-316.

Hutchins M, Thompson SD. 2008. Zoo and aquarium research: priority setting for the coming decades. Zoo Biology 27:488-497.

IUCN. 2012.*The IUCN Red List of Threatened Species: Summary Statistics*. International Union for Conservation of Nature. <http://www.iucnredlist.org/>

IUDZG/CBSG (IUCN/SSC). 1993. Executive Summary of The World Zoo Conservation Strategy: The Role of the Zoos and Aquaria of the World in Global Conservation.

Jurke MH, Czekala NM, Lindburg DG, Millard SE. 1997. Fecal corticoid metabolite measurement in the cheetah (*Acinonyx jubatus*). Zoo Biology 16(2):133-147.

Kagan R, Veasey J. 2010. Challenges of Zoo Animal Welfare. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. D Kleiman, K Thompson, and C Baer. Chicago: The University of Chicago Press.

Keay JM, Singh J, Gaunt MC, Kaur T. 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. Journal of Zoo and Wildlife Medicine 37:234-244.

Kreger MD, Hutchins M. 2010. Ethics of Keeping Mammals in Zoos and Aquariums. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Ladewig J. 2000. Chronic Intermittent Stress: A Model for the Study of Long-term Stressors. In *The Biology of Animal Stress: Basic Principles and Implications for Animal Welfare*. Ed. GP Moberg, and JA Mench. New York: CABI Publishing.

Laikre L. 2010. Genetic diversity is overlooked in international conservation policy implementation. Conservation Genetics 11: 349-354.

Lewis D. 2010. Setting Standards for Evaluation of Captive Facilities: North America. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Liu J, Chen Y, Guo L, Gu B, Liu H, Hou A, Liu X, Sun L, Liu D. 2006. Stereotypic behavior and fecal cortisol level in captive giant pandas in relation to environmental enrichment. Zoo Biology 25:445-459.

Mallapur A, Chellam R. 2002. Environmental influences on stereotypy and the activity budget of Indian leopards (*Panthera pardus*) in four zoos in southern India. Zoo Biology 21:585-595.

Mallapur A, Qureshi Q, Chellam R. 2002. Enclosure design and space utilization by Indian leopards (*Panthera pardus*) in four zoos in southern India. Journal of Applied Animal Welfare Science 5:111-124.

Maple T. 2007. Toward a science of welfare for animals in the zoo. Journal of Applied Animal Welfare Science 10(1):63-70.

Maple TL, Bashaw MJ. 2010. Research Trends in Zoos. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Mattson, MP. 2008. Hormesis and disease resistance: Activation of cellular stress response pathways. Hum Exp Toxicol 27 (2): 155-162.

Mazak V. 1981. *Panthera tigris*. Mammalian Species, No. 152:1-8.

McPhee ME. 2002. Intact carcasses as enrichment for large felids: effects on on- and off-exhibit behaviors. Zoo Biology 21:37-47

McPhee ME, Carlstead K. 2010. The Importance of maintaining Natural Behaviors in Captive Animals. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Mellen J, MacPhee MS. 2001. Philosophy of environmental enrichment: past, present, and future. Zoo Biology 20:211-226.

Mellen JD, Shepherdson DJ. 1997. Environmental enrichment for felids: An integrated approach. International Zoo Yearbook 35(1):191-197.

Mench JA. 1998. Why it is important to understand animal behavior. ILAR Journal 39:20-26.

Moreira N, Brown JL, Moraes W, Swanson WF, Monteiro-Filho ELA. 2007. Effect of housing and environmental enrichment on adrenocortical activity, behavior and reproductive cyclicity in the female tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). Zoo Biology 26:441-460.

Morgan JM, Gramann JH. 1989. Predicting effectiveness of wildlife education programs: a study of students' attitudes and knowledge toward snakes. Wildlife Society Bulletin 12:501-509.

Munson L. 1993. Diseases of captive cheetahs (*Acionyx jubatus*): Results of the cheetah research council pathology survey. Zoo Biology 12:105-124.

Munson L, Nesbit JW, Meltzer DGA, Colly LP, Bolton L, Kriek NPJ. 1999. Diseases of captive cheetahs (*Acionyx jubatus jubatus*) in South Africa: A 20 year retrospective survey. Journal of Zoo and Wildlife Medicine 30:342-347.

Neuringer AJ. 1969. Animals respond for food in the presence of free food. Science 166:399-401.

Newberry RC. 1995. Environmental enrichment: increasing the biological relevance of captive environments. Applied Animal Behaviour Science 44:229-243.

Nowak RM, Paradiso JL. 1983. *Walker's Mammals of the World*, 4th Ed. Baltimore: The Johns Hopkins University Press.

Nowell K, Jackson P. 1996. *Wild cats: status survey and conservation action plan.* Cambridge: International Union for Conservation of Nature.

Ogden J, Heimlich JE. 2009. Why focus on zoo and aquarium education? Zoo Biology 28:357-360.

Palme R. 2005. Measuring fecal steroids: guidelines for practical applications. Annals of the New York Academy of Sciences 1046:75-80.

Patrick PG, Matthews CE, Ayers DF, Tunnicliffe SD. 2010. Conservation and education: prominent themes in zoo mission statements. Journal of Environmental Education 38(3):53-60.

Pitsko LE. 2003. *Wild tigers in captivity: A study of the effects of the captive environment on tiger behavior* (Unpublished master's thesis). Virginia Polytechnic Institute and State University, Blacksburg.

Price MRS. 2005. Zoos as a force for conservation: a simple ambition-but how? Oryx 39:109-110.

Rabb, GB. 2004. The evolution of zoos from menageries to centers of conservation and caring. Curator 47:237-246.

Rands MRW, Adams WM, Bennun L, Butchart SHM, Clements A, Coomes D, Entwistle A, Hodge I, Kapos V, Scharlemann JPW, Sutherland WJ, Vira B. 2010. Biodiversity conservation: challenges beyond 2010. Science 329: 1298-1303.

Reeder DM, Kramer KM. 2005. Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. Journal of Mammalogy 86(2):225-235.

Rees PA. 2003. Asian elephants in zoos face global extinction: should zoos accept the inevitable? Orys 37:20-22.

Rees PA. 2011. *An Introduction to Zoo Biology and Management*. Oxford: Wiley-Blackwell.

Resende LS, Pedretti Gomes KC, Andriolo A, Genaro G, Remy GL, Almeida Ramos V. 2011. Influence of cinnamon and catnip on the stereotypical pacing of oncilla cats (*Leopardus tigrinus*) in captivity. Journal of Applied Animal Welfare Science 14:247-254.

Romero M. 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. General & Comparative Endocrinology 128(1):1-24.

Rothfels N. 2002. *Savages and Beasts: The Birth of the Modern Zoo.* Baltimore: Johns Hopkins University Press.

Routman E, Ogden J, Winsten K. 2010. Visitors, Conservation Learning, and the Design of Zoo and Aquarium Experiences. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Schaller GB. 1972. *The Serengeti Lion: A Study of predator-prey relations*. Chicago: University of Chicago Press.

Selye H. 1936. A syndrome produced by diverse nocuous agents. Nature 138: 32.

Shepherdson D. 2010. Principles of and Research on Environmental Enrichment for Mammals. In *Wild Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.

Shepherdson D, Carlstead K, Mellen JD, Seidensticker J. 1993. The influence of food presentation on the behavior of small cats in confined environments. Zoo Biology 12:203-216.

Shepherdson D, Mellen J, Hutchins M. 1998. *Second Nature: Environmental Enrichment for Captive Animals*. Washington, DC: Smithsonian Institution Press.

Shoemaker AH, Maruska EJ, Rockwell R. 1997. Minimum husbandry guidelines for mammals: Large felids. American Association of Zoos and Aquariums.

Shyne A. 2006. Meta-analytic review of the effects of enrichment on stereotypic behavior in zoo mammals. Zoo Biology 25:317-337.

Skibiel AL, Trevino HS, Naugher K. 2007. Comparison of several types of enrichment for captive felids. Zoo Biology 26:371-381.

St. Aubin D, Ridgway S, Wells R, Rhinehart H. 1996. Dolphin thyroid and adrenal hormones: circulating levels in wild and semidomesticated *Tursiops truncatus*, and influence of sex, age, and season. Marine Mammal Science 12: 1-13.

Stuart C, Stuart T. 2000. *Field Guide to the Larger Mammals of Africa*. Cape Town: Struik.

Sunquist ME, Sunquist FC. 1997. Ecological constraints on predation by large felids. In Seidensticker J, Christie S, Jackson P, editors. Riding the tiger: tiger conservation in human-dominated landscapes. New York: Cambridge Press.

Swaisgood RR, Shepherdson DJ. 2005. Scientific approaches to enrichment and stereotypies in zoo animals: what's been done and where should we go next? Zoo Biology 24:499-518.

Tariff Act, 1930. 19 U.S.C. § 1527 (1996). WestLaw Campus Research. Accessed Apr. 7 2012.

Terio KA, Citino SB, Brown JL. 1999. Fecal cortisol metabolite analysis for noninvasive monitoring of adrenocortical function in the cheetah. Journal of Zoo and Wildlife Mediciate 30(4):484-491.

Terio K, Marker L, Munson L. 2004. Evidence for chronic stress in captive but not free-ranging cheetahs (*Acinonyx jubatus*) based on adrenal morphology and function. Journal of Wildlife Diseases 40(2):259-266.

Touma C, Palme R. 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: The importance of validation. Annals of the New York Academy of Sciences 1046:54-74.

Tribe A, Booth R. 2003. Assessing the role of zoos in wildlife conservation. Human Dimensions of Wildlife 8:65-74.

United Nations. 1992. *Convention on Biological Diversity*. Rio de Janeiro. <http://www.cbd.int/doc/legal/cbd-en.pdf>

United Nations. 2007. *The Millennium Development Goals Report*. New York. <http://www.un.org/millenniumgoals/pdf/mdg2007.pdf>

USA Travel Guide. 2009. *The Top 10 Zoos in America.* <www.wrsol.com/usatravelguide/>

Van Metter JE, Harriger MD, Bolen RH. 2008. Environmental enrichment utilizing stimulus objects for African lions (*Panthera leo leo*) and Sumatran tigers (*Panthera tigris sumatrae*). BIOS 79(1):7-16.

Van Praag H, Kempermann G, Gage FH. 2000. Neural consequences of environmental enrichment. Nature Reviews: Neuroscience 1:191-198.

WAZA. 2005. Building a Future for Wildlife - The World Zoo and Aquarium Conservation Strategy.

WCS. 2012. *About Us.* Wildlife Conservation Society. < http://www.wcs.org/about-us.aspx>

Wells DL, Coleman D, Challis MG. 2006. A note on the effect of auditory stimulation on the behaviour and welfare of zoo-housed gorillas. Applied Animal Behaviour Science 100:3-4.

Wells DL, Egli JM. 2004. The influence of olfactory enrichment on the behavior of captive black-footed cats, *Felis nigripes*. Applied Animal Behaviour Science 85:107-119.

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

Wielebnowski N. 2003. Stress and distress: Evaluating their impact for the well-being of zoo animals. Journal of American Veterinary Medical Association 223(7):973-976.

Wielebnowski N, Watters JV. 2007. Applying fecal endocrine monitoring to conservation and behavior studies of wild mammals: Important considerations and preliminary tests. Israel Journal of Ecology & Evolution 53(1):439-460.

Wielebnowski N. RE: University of Maryland Undergraduate Research [electronic mail on the Internet]. Message to: Team BLAZE. 2009 Oct. 9 [cited 2009 Oct. 9]. [about 3 paragraphs].

Wilson EO. 2000. *Sociobiology: The New Synthesis*. Cambridge, MA: Harvard University Press.

Yerkes RM. 1925. *Almost Human*. New York: The Century Co.

Young KM, Walker SL, Lanthier C, Waddell WT, Monfort SL, Brown JL. 2004. Noninvasive monitoring of adrenocortical activity in carnivores by fecal glucocorticoids analyses. General and Comparative Endocrinology 137:148-165.

Zimmerman A. 2010. The Role of Zoos in Contributing to *In Situ* Conservation. In Wild *Mammals in Captivity: Principles and Techniques for Zoo Management*. Ed. DG Kleiman, KV Thompson, and C Kirk Baer. Chicago: The University of Chicago Press.