Regis University

ePublications at Regis University

Regis University Student Publications (comprehensive collection) | Regis University Student Publications

Spring 2018

MS Environmental Biology Capstone Project

Taylor Readyhough

Follow this and additional works at: https://epublications.regis.edu/theses

Part of the Behavior and Ethology Commons, Ornithology Commons, Poultry or Avian Science Commons, and the Zoology Commons

Recommended Citation

Readyhough, Taylor, "MS Environmental Biology Capstone Project" (2018). *Regis University Student Publications (comprehensive collection)*. 853.
https://epublications.regis.edu/theses/853

This Thesis - Open Access is brought to you for free and open access by the Regis University Student Publications at ePublications at Regis University. It has been accepted for inclusion in Regis University Student Publications (comprehensive collection) by an authorized administrator of ePublications at Regis University. For more information, please contact epublications@regis.edu.

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Taylor S. Readyhough

A Project Presented in Partial Fulfillment
of the Requirements for the Degree
Masters of Science
in Environmental Biology

REGIS UNIVERSITY
May, 2018

MS ENVIRONMENTAL BIOLOGY
CAPSTONE PROJECT

by

Taylor S. Readyhough

has been approved

May, 2018

APPROVED:

_____________________________________, Amy Schreier, Ph.D. (Faculty Advisor)

_____________________________________, Amy Schreier, Ph.D. (Chapters 1 & 2)

_____________________________________, Kristofor Voss, Ph.D. (Chapter 3)

_____________________________________, Mike Ghedotti, Ph.D. (Chapter 4)

_____________________________________, Ariel Wooldridge, M.S. (Exit Survey & Repository)

Table of Contents

TABLES AND FIGURES

Chapter 1, List of Tables

Chapter 1, List of Figures

Chapter 3, List of Tables

Chapter 3, List of Figures

CHAPTER 1. LITERATURE REVIEW: THE IMPORTANCE OF ARTIFICIAL LIGHT ENVIRONMENTS FOR CAPTIVE BIRDS

Light is a major force driving life on our planet. Without light, we would not have an atmosphere, or aquatic and terrestrial biomes. Most life cycles on Earth depend on light patterns, and light plays many roles in the biotic world (Hartman 1998). Many birds specifically use light and photoperiods to dictate their annual reproductive cycles, migration, and their daily activity patterns (de Jong et al. 2016; Dominoni et al. 2016; Poot et al. 2008; Raap et al. 2016; Raap et al. 2017; Turek et al. 1976), which can be affected by exposure to artificial light. The extended time that captive birds spend in artificially lit environments may have a negative effect on their annual cycles and daily activity patterns, therefore, light is an important factor to consider when managing captive birds. In order to minimize the negative effects of artificially lit environments, animal management teams should incorporate opportunities and spaces that allow for sunning behaviors, include more natural light sources, and manage artificial light cycles to mirror natural cycles. By integrating light management into management plans for captive birds, we can improve their health and promote successful reproduction.

Light influences animals' physical health. Most animals require light, and the heat affiliated with it, in order to develop properly. Currently, most of the research in this area occurs in agricultural science. For example, studies show that piglets are more likely to survive if they are exposed to heating lamps after birth (Zhou et al. 1999). The energy from heating lamps reduces the amount of energy that the piglets have to produce on their own, thereby reducing the metabolic stress they endure. Farmers frequently use artificial light to promote development in chicks (Griffin and Vardaman 1971). Because of the increased temperatures and light produced by the heat lamps, the chicks develop faster and have increased metabolic activity (Griffin and Vardaman 1971). In agriculture, many young animals benefit from this metabolic support.

Later in life, many animals use light exposure as a mechanism for promoting healthy body condition. Amphibians and reptiles use light for thermoregulation; captive turtles and lizards need the heat from artificial lights just like chicks do (Dubois et al. 2008). Some small mammals need light to thermoregulate as well (Rand 1935; Wacker et al. 2017). Numerous bird species show the same propensity for thermoregulation via light sources (Blem and Blem 1992; Leck 1974; Rogers 1976). In these cases, light essentially serves the same role as it does in the young animals; light reduces the metabolic stress of thermoregulation. Heat lamps and light sources are carefully considered during reptile and amphibian enclosure designs (Baines, F. M. 2009), but they are frequently disregarded in avian exhibits because they are not as obviously affiliated with thermoregulation.

Figure 1: *Black noddies sunning themselves on the sand (getinthehotspot.com). Research indicates that this sunning behavior acts to significantly reduce the presence of chewing lice on the noddies' feathers.*

Birds have numerous biological relationships with light. On the one hand, many bird species engage in sunning behaviors to reduce metabolic strain and maintain body condition.

When birds maintain homeostasis, they frequently sit in the sun in order to increase their body temperatures rather than expend valuable energy to raise their internal temperatures (Leck 1974). On the other hand, some birds use sunlight to decrease ectopic parasites (Moyer and Wagenbach 1995). One study showed that by exposing their feathers to sunlight for an extended time, black noddies reduce the number of chewing lice parasites by approximately 50% (Figure 1). Simply by increasing the temperature across their feathers, black noddies could significantly impact the amount of parasitism they experience. This shows how birds utilize extended exposure to sunlight during the day in order to promote their own health (Moyer and Wagenbach 1995). Purposeful use of artificial light sources, such as UV lights in avian exhibits, can greatly benefit birds even if they do not immediately depend on the light sources for survival or development.

In order to successfully manage captive bird populations, we must offer ample opportunities for sunning behaviors. If the birds are housed indoors, then keepers could install UV lights inside their enclosure in order to simulate the UV-rays from the sun. This would provide a set area within the enclosure to support the birds' natural sunning behaviors. However, this is not an equivalent replacement for natural sunlight and it includes various potential drawbacks. For example, there would be a high associated financial cost with UV-lamps and they do not necessarily produce consistent heat, even within lamps from the same manufacturer (Galama et al. 2002).

The presence or absence of light (both natural and artificial) at night is also an important factor for birds. Migratory birds rely on very low light levels in order to navigate at night (Poot et al. 2008). When nocturnally-migrating birds encounter unnatural amounts of light at night, they frequently become disoriented. This can lead to a failure to successfully navigate to their original destination, or to their injury and/or death. A study showed that the color of artificial light that migrating birds encounter directly impacts their navigation. Red and white lights confused and attracted birds more often, while green lights showed the lowest impacts on the nocturnally-migrating birds (Poot et al. 2008).

In humans, night shift workers produce lower levels of melatonin and also have higher rates of cancer, diabetes, and reproductive issues (Davis et al. 2001; Eastman and Martin 1999). Since melatonin can affect many biological processes, it is important to consider the impacts of light exposure in captive environments on melatonin levels in the animals. Research showed that the continued exposure to artificial light (especially blue light) reduces melatonin levels in captive pygmy slow lorises (Fuller 2014). By monitoring melatonin in pygmy slow lorises' saliva, Fuller (2014) showed that when they were exposed to blue light, the melatonin levels in their saliva decreased. Because melatonin is important in reproduction and sleep, lowered levels of melatonin can lead to huge negative shifts in these cycles.

Integrating more natural light into captive birds' enclosures could improve their overall condition. Because we still understand very little about the impacts that sunlight has on birds, we can attempt to replicate a natural environment but we will most likely fall short. Instead of attempting to fully understand the important factors that ought to be included, the birds' enclosures could be built with the goal of integrating more natural sunlight (Galama et al. 2002). This sunlight also provides sunning opportunities for the birds which would have its own set of benefits including metabolic support and parasite reduction. Some drawbacks affiliated with this approach include the prohibitive cost of remodeling current exhibits, and the fact that daily light cycles differ greatly across the globe. If a bird evolved in a tropical environment with limited daily shifts in the light cycle, and then it is exposed to extreme daily light shifts in a zoo in the northern hemisphere, it will not necessarily benefit as much as a bird that evolved in an environment similar to the one at the zoo (Galama et al. 2002). However, the birds still receive the cumulative benefits from natural light exposure.

Another role light plays in animals' lives comes in the form of photoperiods, the length of time that organisms experience sunlight each day. Photoperiods are major regulators of circadian rhythms throughout animals' lives (Elliott, J. A. 1976). When birds experience artificial light at night, it directly affects the amount of sleep they get (Raap et al. 2017). Free-living great tits lost approximately 40 minutes of sleep each night that they experienced artificial light within their nest box for the duration of the night. Specifically, the birds fell asleep approximately 12 minutes later during the time when they experienced artificial light (Raap et al. 2017; Table 1).

Sleep parameter	Control	Light effect
Sleep onset	4.15 (−1.19, 9.49)	16.20 (8.92, 23.50)
Awakening time	−26.48 (−35.50, −17.50)	−24.10 (−31.70, −16.60)
Leaving time	−19.54 (−27.70, −11.30)	−17.70 (−24.62, −10.80)
Evening latency	1.57 (1.32, 1.81)	0.60 (0.36, 0.78)
Morning latency	1.32 (0.86, 1.77)	0.70 (0.20, 1.13)
Time on entrance	−0.28 (−1.22, 0.66)	1.10 (0.26, 1.92)
Nr on entrance	0.57 (0.04, 1.11)	1.30 (0.88, 1.72)
Sleep duration	844 (833, 855)	−40.20 (27.12, 53.20)
Sleep duration/night duration	1.01 (1.00, 1.03)	0.05 (0.03, 0.06)
Sleep bout length	10.14 (8.12, 12.20)	2.70 (1.23, 4.19)
Sleep bout/h	5.33 (4.37, 6.28)	−1.30 (−2.05, −0.49)

Table 1: *From Rapp et al. (2016) showing the major findings of their study on light effects on birds' sleep. The most important findings for this review are highlighted with a yellow line: artificial light delayed sleep onset by approximately 12 minutes and decreased sleep duration by approximately 40 minutes.*

Melatonin is an important biological compound involved in sleep and reproductive cycles (Turek et al. 1976) that is directly affected by light levels. Because melatonin is produced in much larger amounts at night, shifts in nighttime light exposure specifically can greatly influence melatonin production. Raap et al. (2016) showed that increased exposure to artificial light at night caused decreases in nitric oxide levels and increases is haptoglobin level in free-living nestling great tits' blood. This biochemical shift indicates an immune response in the nestlings that uses up valuable energy and resources within their growing bodies. Decreased melatonin levels frequently lead to similar shifts in haptoglobin and nitric oxide levels, so these results may indicate decreased melatonin production in the great tit nestlings (Raap et al. 2016). Because light plays such a crucial role in animals' lives, exposure to excess light may affect physical health, development, and circadian rhythms in animals.

There is increasing evidence that artificial light at night directly affects birds' activity levels, both in the wild and in captivity. De Jong et al. (2016) conducted a controlled experiment that illustrates the importance of the amount of artificial light that birds encounter. They showed a strong dose-dependent relationship between great tits' activity levels and the intensity of the light they experienced at night. The more intense the light was at night, the more active the great tits were throughout the day and night (de Jong et al. 2016). The great tits also had lower melatonin levels during the nights when they experienced the most intense light (de Jong et al. 2016). It is important to note that exposure to artificial light at night influenced the birds' behaviors throughout their daily cycles, not only during the period of light exposure.

When captured European blackbirds experienced artificial light at night, their reproductive systems developed almost a month earlier than blackbirds that did not encounter artificial light (Dominoni et al. 2016). This study showed that shifts in daily light cycles signal changes in birds' circadian rhythms that result in reproductive development. Therefore, artificial light causes adjustments to daily light cycles that also lead to altered reproductive development (Dominoni et al. 2016). This shift may be important to consider in captive breeding programs because the timing of birds' reproductive development can impact reproductive success (Verhulst and Nilsson 2008). If artificial light exposure alters captive birds' daily patterns, improper management of their light environment could drastically affect their health. Because sleep plays a crucial role in maintaining brain-chemistry and supporting physical health in all animals, any changes to birds' sleep patterns could have major implications. This is especially important for captive birds whose daily patterns must shift in order to match seasonal changes in zoo business-operating hours.

When more natural light cannot easily be integrated into enclosures, purposeful artificial light pattern manipulation may aid in promoting captive birds' health and reproductive success. By researching the specific birds' habitats and daily light cycles, keepers could work to mimic these patterns (Galama et al. 2002). This would serve to create a more natural environment for the birds and might work to minimize any negative light effects that we do not yet understand. Theoretically, these patterns could be manipulated within each exhibit in order to create the most natural light cycles for the specific inhabitants of the area.

When birds are in a captive environment, light remains an important driver of their biology. However, that light does not always follow natural patterns and intensities because any non-native birds should be housed indoors (Irwin et al. 2013). Captive birds must be permitted to engage in sunning behaviors in order to reduce metabolic strains on their systems and to reduce parasites (Blem and Blem 1992; Leck 1974; Rogers 1976; Moyer and Wagenbach 1995). By installing UV lamps inside of birds' enclosures, we can provide an opportunity for them to engage in their natural sunning behaviors (Galama et al. 2002). Incorporating more natural sunlight into enclosures can benefit birds by introducing natural photoperiods. Additionally, light has direct effects on development and circadian rhythms in birds (Gwinner et al 1997; Galama et al. 2002; Raap et al. 2016; Raap et al. 2017). Artificial light can have major positive and negative impacts on captive birds' health and reproductive success; therefore, it must be considered of utmost importance in captive bird management. In order to improve the health of captive birds, and to increase the birds' reproductive success, avian exhibits should include opportunities for sunning behaviors, incorporate more natural light sources, and manipulate artificial light cycles to mirror natural cycles.

References

Candy, A. (2012). *Black noddy terns sunning themselves in a clearing in the Pisonia forest on Heron Island.* [JPG]. Retrieved September 26, 2017 from getinthehotspot.com

Baines, F. M. (2009). Reptile lighting information. *Reptiles Magazine*. Retrieved September 26, 2017 from www.reptilesmagazine.com/Reptile-Health/Habitats-Care/Reptile-Lighting-Information

Blem, C. R., & Blem, L. B. (1992). Some observations of sunbathing in swallows (Algunas observaciones sobre baños de sol en golondrinas). *Journal of Field Ornithology, 63*(1), 53-56.

Davis, S., Mirick, D. K., & Stevens, R. G. (2001). Night shift work, light at night, and risk of breast cancer. *Journal of the National Cancer Institute*, *93*(20), 1557-1562.

de Jong, M., Jeninga, L., Ouyang, J. Q., van Oers, K., Spoelstra, K., & Visser, M. E. (2016). Dose-dependent responses of avian daily rhythms to artificial light at night. *Physiology & Behavior*, *155*, 172-179.

Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1756), e20123017.

Dubois, Y., Blouin-Demers, G., & Thomas, D. (2008). Temperature selection in wood turtles (Glyptemys insculpta) and its implications for energetics. *Écoscience*, *15*(3), 398-406.

Eastman, C. I., & Martin, S. K. (1999). How to use light and dark to produce circadian adaptation to night shift work. *Annals of Medicine*, *31*(2), 87-98.

Elliott, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. *Federation Proceedings, 35*(12), 2339-2346.

Fuller, G. A. (2014). *The night shift: lighting and nocturnal strepsirrhine care in zoos* (Doctoral dissertation, Case Western Reserve University).

Galama, W., King, C., & Brouwer, K. (2002). EAZA hornbill management and husbandry guidelines. *Artis Zoo, The Netherlands: National Foundation for Research in Zoological Gardens*.

Griffin, J. G., & Vardaman, T. H. (1971). Effects of radiant heat on market-size broiler chicks grown in a cyclic high temperature environment. *Poultry Science*, *50*(2), 459-463.

Gwinner, E., Hau, M., & Heigl, S. (1997). Melatonin: generation and modulation of avian circadian rhythms. *Brain Research Bulletin*, *44*(4), 439-444.

Hartman, H. (1998). Photosynthesis and the origin of life. *Origins of Life and Evolution of the Biosphere*, *28*(4-6), 515-521.

Irwin, M. D., Stoner, J. B., & Cobaugh, A. M. (Eds.). (2013). *Zookeeping: an introduction to the science and technology*. University of Chicago Press.

Leck, C. F. (1974). Sunbathing in the streamertail hummingbird. *The Wilson Bulletin*, *86*(1), 78-79.

Moyer, B. R., & Wagenbach, G. E. (1995). Sunning by black noddies (Anous minutus) may kill chewing lice (Quadraceps hopkinsi). *The Auk*, *112*(4), 1073-1077.

Poot, H., Ens, B., de Vries, H., Donners, M., Wernand, M., & Marquenie, J. (2008). Green light for nocturnally migrating birds. *Ecology and Society*, *13*(2), 47.

Rand, A. L. (1935). On the habits of some Madagascar mammals. *Journal of Mammalogy*, *16*(2), 89-104.

Raap, T., Casasole, G., Pinxten, R., & Eens, M. (2016). Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds. *Environmental Pollution*, *218*, 909-914.

Raap, T., Sun, J., Pinxten, R., & Eens, M. (2017). Disruptive effects of light pollution on sleep in free-living birds: season and/or light intensity-dependent?. *Behavioural Processes, 144,* 13-19.

Rodgers, J. A. (1976). Spread-wing sunbathing by juvenile white-faced ibis. *The Auk*, *93*(2), 375-376.

Turek, F. W., McMillan, J. P., & Menaker, M. (1976). Melatonin: effects on the circadian locomotor rhythm of sparrows. *Science*, *194*(4272), 1441-1443.

Verhulst, S., & Nilsson, J. Å. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *363*(1490), 399-410.

Wacker, C. B., McAllan, B. M., Körtner, G., & Geiser, F. (2017). The role of basking in the development of endothermy and torpor in a marsupial. *Journal of Comparative Physiology B*, 1-10.

Zhou, H., & Xin, H. (1999). Effects of heat lamp output and color on piglets at cool and warm environments. *Applied Engineering in Agriculture*, *15*(4), 327.

CHAPTER 2. GRANT PROPOSAL: A NOT-SO-SILENT NIGHT: INVESTIGATING THE EFFECTS OF EXTENDED ZOO OPERATING HOURS DURING "ZOO LIGHTS" ON A PAIR OF GREAT INDIAN HORNBILLS

Abstract

Life in a zoo brings a score of stressors into the lives of captive animals, including artificial light, crowds of visitors, and increased noise levels. Stress especially impacts captive birds, and continued exposure to these stressors can negatively affect birds' reproductive success and overall well-being. Staff at the Denver Zoo noticed increased aggression between a male and female pair of great Indian hornbills during the winter of 2016. This behavioral shift coincided with Zoo Lights, a holiday event that results in the hornbills' exhibit remaining open to the public for approximately four extra hours through the entire month of December. Additionally, the hornbills are especially sensitive to stress during the winter because it coincides with their breeding season. With this study, I plan to develop a behavioral profile of the pair of hornbills during three time periods: prior to Zoo Lights, during Zoo Lights, and after Zoo Lights. I will then compare the three time periods in order to examine the effects of the extended operating hours on the hornbills' behavior. My results will provide the Denver Zoo with insights into the hornbills' behaviors and will support animal-care recommendations to reduce their stress during Zoo Lights.

Background/Rationale/Significance

Captivity exposes animals to novel experiences and stressors. In captive conditions, they frequently encounter artificial light, crowds of people, and elevated noise levels compared to natural environments. These factors are potential sources of stress for captive animals, and can especially impact captive birds (Dickens and Bentley 2014; Owen et al. 2004; Terio et al. 2004). Reducing captive birds' stress is important since they are often a part of captive breeding and conservation programs that are crucial for the continuation of their species (Conway 2003).

The importance of light for most life-forms cannot be understated. In birds, light regulates sleep patterns and reproductive cycles, and daily light cycles determine the behaviors of birds and many other animals (de Jong et al. 2016; Dominoni et al. 2016; Poot et al. 2008; Raap et al. 2016; Raap et al. 2017; Turek et al. 1976). In captivity, light levels are still crucial to birds' biology, but the light they experience is not always natural. Most indoor exhibits involve artificial light sources; zoos house exotic birds indoors to control the temperature and humidity levels in their enclosures. This continuous exposure to artificial light can alter captive birds' sleep and activity patterns. In free-living great tits, more artificial light at night caused shifts in biochemical levels in the birds' blood (Raap et al. 2016). This indicated an immune response that used up valuable energy and resources within the nestling birds' growing bodies.

Light levels regulate birds' activities each day and may trigger their annual reproductive periods as well (Elliott 1976). Photoperiods, the length of time that animals experience sunlight each day, also affect animals. Photoperiods regulate animals' circadian rhythms throughout their lives (Elliott 1976). Raap et al. (2017) showed that when birds experienced artificial light at night, it directly impacted the amount of sleep they got. Great tits lost approximately 40 minutes of sleep when they were exposed to artificial light inside their nest boxes all night (Raap et al. 2017). In another study, the more intense the light was at night, the more active great tits were throughout the day and night (de Jong et al. 2016). When European blackbirds experienced artificial light at night, their reproductive systems developed almost a month earlier than blackbirds that did not experience artificial light (Dominoni et al. 2016). Therefore, artificial light causes adjustments to daily light cycles that also lead to altered reproductive development (Dominoni et al. 2016). This shift may be important to consider in captive breeding programs because the timing of birds' reproductive development can impact reproductive success (Verhulst and Nilsson 2008). The extended time that captive birds spend in artificially lit environments may have a negative effect on their annual cycles and daily activity patterns; therefore, light is an important factor to consider when managing captive birds.

Captivity forces animals to remain in close proximity to crowds of humans, which can be a stressor for the animals (Morgan and Tromborg 2007). One study showed that citron-crested and Moluccan cockatoos changed their behavior when there were children close to the birds' enclosures (Collins and Marples 2015). The citron-crested cockatoos increased their social behaviors while the Moluccan cockatoos retreated from the children (Collins and Marples 2015). This study illustrates the importance of understanding species-specific responses to crowds in captive birds. Additionally, crowds of people also bring high noise levels that can exacerbate this already stressful situation. In many captive birds, stress reveals itself through feather-damaging behaviors, a disinterest in novel experiences, and a lack of exploratory behaviors (Fox 1968; King 1993; Cockrem 2007). Reducing stress levels in captive birds can improve their welfare and encourage successful breeding in the cases when zoos are working to save their declining populations.

Hornbills are an endangered family of birds known as *Bucerotidae* that are distinguished by their large casque, a keratinous structure that sits on top of their hefty bill. Logging operations in hornbills' natural habitat are leading to the rapid decline of their wild populations. There are 54 known species of hornbills today (Kemp 1993). The great Indian hornbill is one of the larger species of hornbills (Kemp 1993). Hornbills are sexually dimorphic; female great Indian hornbills are smaller than males and the males have dark-red eyes, whereas the females have light-blue eyes.

In the wild, hornbills reside in Africa and Southeast Asia where their habitat may consist of forests, rainforests, and savannahs (Kemp 1993). The great Indian hornbill inhabits China, India, Bhutan, Thailand, Laos, and occasionally a few other Asian countries (Kemp 1993). They reside in old growth tropical forests, which are being reduced by logging operations (de Ruiter 1998). It is increasingly important to develop successful captive breeding programs in order to support research and conservation efforts for these magnificent birds.

Hornbills build their nests in cavities in trees (Poulsen 1970; James and Kannan 2007). Together, the mating pair seals the female into the nest with a mixture of fecal matter and mud for the duration of the incubation period in a process referred to as “mudding in.” They leave a small opening in the nest entrance through which the male passes food and the female defecates (Poulsen 1970; James and Kannan 2007). Hornbills are notoriously sensitive breeders; this may be due to the fact that the female must remain in such a vulnerable position for a long period of time (Galama et al. 2002). Therefore, any increases to stress during their breeding season can result in failed reproductive attempts or a lack of interest in reproducing at all.

Each December, the Denver Zoo hosts Zoo Lights, an entertainment event that includes large holiday light displays, shows, and access to some of the animal exhibits (denverzoo.org). The animal exhibits remaining open to guests during Zoo Lights results in extended exposure to artificial light, crowds, and noise for the animals. In 2017, Zoo Lights will occur daily from 5:30-9:00pm, which equates to approximately four extra hours of exposure to potential stressors for the animals. The Denver Zoo is interested in assessing whether these extended operating hours have negative effects on the animals in the exhibits that remain open. This study will focus on a pair of great Indian hornbills (*Buceros bicornis*) that reside in the Toyota Elephant Passage exhibit. The great Indian hornbills' zookeepers noticed dramatic behavioral changes during December of last year. They reported that the male was increasingly aggressive towards the female, chasing and biting her so frequently that it was difficult for her to rest in any one place for more than a few minutes. The zookeepers suspect this aggression may be related to the extended light exposure because the behavioral changes closely matched the timeline of Zoo Lights (Vyas pers. comm.). The impacts from Zoo Lights are exceptionally important during this time of year because the winter coincides with the hornbills' breeding season. The increased stress and related aggression may have direct impacts on the hornbill pair's mating success.

This study will test the zookeepers' anecdotes about increased aggression during Zoo Lights. They believe this behavioral shift may be due to increased reproductive hormones in the male great Indian hornbill from the excess exposure to light at night. Since successful reproduction may not occur under stress, it is beneficial to develop a stronger understanding of these specific hornbills' behavioral patterns under increased stress conditions during Zoo Lights.

This research aligns with the Regis University mission because it will provide a recommendation that will improve the lives of the captive great Indian hornbills and can open up investigation into the welfare of other animals. Furthermore, this research examines the responsibility humans have to protect the natural environment and to treat other living creatures with respect. There are benefits to keeping animals in captivity, but it is our social responsibility to ensure that we reduce the potentially negative consequences for the captive animals as much as we possibly can.

Purpose and Specific Aims

This study will examine how the extended zoo-operating hours during Zoo Lights at the Denver Zoo affect the great Indian hornbills' behavior. Since the great Indian hornbills' exhibit is open to the public during Zoo Lights, they experience extended exposure to artificial light, crowds, and noise throughout the month of December. These stressors may cause changes in sleep patterns and reproductive cycles that could lead to behavioral changes in the hornbills during this month. Results from this research could lead to changes in how these animals are housed and when they are placed on exhibit. These results will also guide a recommendation for the Denver Zoo about keeping some exhibits open late during Zoo Lights.

Due to the extended zoo hours during Zoo Lights, I expect the great Indian hornbills to be more active during the day in December compared to the pre- and post-Zoo Lights periods because they will experience more artificial light at night and crowds of people will still visit their exhibit until approximately 9:00pm. On the other hand, since Zoo Lights represents a significant shift in daily routines that may be a stressor in-and-of-itself, I expect the hornbills to show decreased active and exploratory behaviors in the evenings during Zoo Lights in comparison to their normal daytime behaviors during the Zoo Lights time period. Furthermore, the increased exposure to artificial light and crowds will cause the male great Indian hornbill to exhibit increased aggressive and chasing behaviors towards the female during December compared to the pre- and post-Zoo Lights periods. Since increased day-length may be one of the factors that triggers reproductive cycles in hornbills, the great Indian hornbills will spend more time inside the nest box or within 1 meter of the nest box in December compared to the pre- and post-Zoo Lights observational periods.

Methods

Study Site

The great Indian hornbills reside in Toyota Elephant Passage at the Denver Zoo. Originally, their enclosure was made for bats but has since been modified to provide a habitat for the hornbills. They are the only species in their enclosure. It includes several trees with branches and ropes for them to use as perches, however, there is no green, leafy vegetation around them. There is a nesting box in the front-left corner of the enclosure and during their mating season (winter), the zookeepers provide tubs with mud for them to use to “mud-in” the female. They receive natural light through four circular skylights in the roof and there are numerous other artificial light bars that illuminate the enclosure.

Data Collection

I will collect data throughout the week during times that the zoo staff have designated as “slow”, “average”, and “busy” regarding patron attendance. I will collect data at least five times a week, for one hour each time. In order to compare the hornbills’ behaviors, I will collect data during three periods: before (October/November), during (December), and after Zoo Lights (January/February). At the end of my study, I will have approximately 25 hours of observational data from each sampling period (75 hours total).

I will collect data on the behavior of both the male and female individuals over 1-hour sampling periods, recording activity data (Appendix A) every minute using instantaneous scan sampling (Altmann 1974). In addition to the behavioral data, I will also collect 0/1 data for the birds’ proximity to each other (1 = within 1m of each other, 0 = more than 1m from each other) and the nest (1 = within 1m of the nest, 0 = more than 1m from the nest). Crowd and noise data will also be collected at each 1-minute interval. Crowd size will be broken down into categories (low = 1-4 people, medium = 5-8 people, and high = 9+ people). I will also use three categories to estimate noise level (low = most normal background noises are audible, medium = only louder background noises are audible, and high = no normal background noises are audible over the crowd noise) during the same time intervals.

I will coordinate with the zookeepers to minimize the impact of their work on my study. When a zookeeper enters the exhibit, hornbill behavior changes drastically because the keepers are associated with food and other beneficial interactions. Therefore, I will work around the keepers' schedules to observe the hornbills when they will not need to enter the enclosure. In the event that the keepers do enter during my collection times, that time period plus one minute after the keeper has left and cannot be heard near the exhibit anymore will be eliminated from data analysis. This will allow the birds to calm down after the keeper leaves the exhibit, reducing the potential bias of the keeper interactions.

I will work closely with Katie Vyas, the Assistant Curator of Birds, to help determine which behaviors indicate stress. I will categorize the hornbills' behaviors into resting or active behaviors. Within the active category, there will be two subcategories: aggressive and non-aggressive (Appendix A). Much of the time, behaviors like chasing and neck biting can be indicative of stress but can also be associated with courtship and mating behaviors (Vyas, pers. comm.). For this study, I will consider these behaviors to be aggressive.

Data Analysis

In order to test my hypotheses, I will use various data analysis techniques within the R data analysis software (R version 3.4.1, R Core Team 2017). I will compare the hornbills' activity levels across my three sampling periods in order to determine if the hornbills are more active during the day in December and less active during the Zoo Lights evenings. I will examine if there are any significant differences in activity level between the three periods through an ANOVA. I will compare the amount of chasing and aggressive behaviors that the male exhibits during each sampling period in order to test whether these behaviors increase during Zoo Lights. I will use a linear model and a generalized linear hypothesis test to determine if there is a significant difference between the three periods. Finally, I will use a generalized linear model to examine if there is a steady increase (and then decrease) in the amount of time both birds spend near/in the nest box over the three time periods.

Work Plan

10/15/2017-11/25/2017 First data collection period (~25 hrs)

12/01/2017-12/31/2017 Second data collection period (~25 hrs during Zoo Lights + ~10 hrs during the day)

01/15/2017-02/15/2017 Third data collection period (~25 hrs)

02/15/2017-03/15/2017 Data analysis

03/15/2017-04/15/2017 Paper write-up and submission

Application to Current Coursework

This study serves as the focal point for my MS in Environmental Biology capstone project and involves numerous skills from my graduate coursework. Specifically, I will use sampling protocols from Advanced Behavioral Ecology, data analysis techniques from Environmental Biostatistics and Research Design, and data modeling from Advanced Ecology and Modeling. Additionally, this grant proposal highlights the professional writing skills I am gaining from my Environmental Biology Colloquium and Grant Writing Seminar.

References

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3), 227-266.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(2), 169-178.

Collins, C. K., & Marples, N. M. (2015). Zoo playgrounds: a source of enrichment or stress for a group of nearby cockatoos? A case study. *Journal of Applied Animal Welfare Science*, *18*(4), 375-387.

Conway, W. (2003). The role of zoos in the 21st century. *International Zoo Yearbook*, *38*(1), 7-13

de Ruiter, M.(1998). The great Indian hornbill a breeding attempt. *AFA Watchbird*, 25(1), 34-35.

de Jong, M., Jeninga, L., Ouyang, J. Q., van Oers, K., Spoelstra, K., & Visser, M. E. (2016). Dose-dependent responses of avian daily rhythms to artificial light at night. *Physiology & Behavior*, *155*, 172-179.

Dickens, M. J., & Bentley, G. E. (2014). Stress, captivity, and reproduction in a wild bird species. *Hormones and Behavior*, *66*(4), 685-693.

Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1756), e20123017.

Elliott, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. *Federation Proceedings, 35*(12), 2339-2346.

Fox, M. W. (1968). *Abnormal behavior in animals.* Philadelphia: Saunders (W.B.) Co. Ltd.

Fuller, G. A. (2014). *The night shift: lighting and nocturnal strepsirrhine care in zoos* (Doctoral dissertation, Case Western Reserve University).

Galama, W., King, C., & Brouwer, K. (2002). EAZA hornbill management and husbandry guidelines. *Artis Zoo, The Netherlands: National Foundation for Research in Zoological Gardens*.

Gwinner, E., Hau, M., & Heigl, S. (1997). Melatonin: generation and modulation of avian circadian rhythms. *Brain Research Bulletin*, *44*(4), 439-444.

James, D. A., & Kannan, R. (2007). Wild Great Hornbills (*Buceros bicornis*) do not use mud to seal nest cavities. *Wilson Journal of Ornithology*, *119*(1), 118-121.

Kemp, A.C. (1993) Conservation of Asian hornbills and their habitats: an introduction, *Manual to the Conservation of Asian Hornbills* (Editors: P. Poonswad and A.C. Kemp), Hornbill Project, Mahidol University Bangkok, 1993, pp. 4-23.

King, C. E. (1993). Environmental enrichment: is it for the birds? *Zoo Biology*, *12*(6), 509-512.

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, *102*(3), 262-302.

Owen, M. A., Swaisgood, R. R., Czekala, N. M., Steinman, K., & Lindburg, D. G. (2004). Monitoring stress in captive giant pandas (Ailuropoda melanoleuca): behavioral and hormonal responses to ambient noise. *Zoo Biology*, *23*(2), 147-164.

Poot, H., Ens, B., de Vries, H., Donners, M., Wernand, M., & Marquenie, J. (2008). Green light for nocturnally migrating birds. *Ecology and Society*, *13*(2).

Poulsen, H. (1970). Nesting behaviour of the black-casqued hornbill *Ceratogymna atrata* (Temm.) and the great hornbill *Buceros bicornis. Ornis Scandinavica*, *1*(1), 11-15.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Raap, T., Casasole, G., Pinxten, R., & Eens, M. (2016). Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds. *Environmental Pollution*, *218*, 909-914.

Raap, T., Sun, J., Pinxten, R., & Eens, M. (2017). Disruptive effects of light pollution on sleep in free-living birds: season and/or light intensity-dependent? *Behavioural Processes, 144,* 13-19.

Terio, K. A., 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.

Turek, F. W., McMillan, J. P., & Menaker, M. (1976). Melatonin: effects on the circadian locomotor rhythm of sparrows. *Science*, *194*(4272), 1441-1443.

Verhulst, S., & Nilsson, J. Å. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *363*(1490), 399-410.

Appendix A- Hornbill Ethogram

Behavior	Definition	Code
Resting Behaviors		
Resting	Sitting upright on a perch with no movement of body; small head movements may be noted	r
Vigilant	Watching the surroundings with interest	v
Active- Non-Aggressive Behaviors		
Stretching	Body is stationary but with significant head/wing/limb movements	str
Eating	Placing bill in food bowl to retrieve items and ingesting those items	e
Flying	Using wings to move from one location to another	f
Hopping	Moving along the length of a perch in a hopping motion	h
Object manipulation	Using bill to make contact with an inanimate object	om
Bill rub	Rubs either side of bill along a perch in a sweeping motion	br
Vocalize	Any vocalization from birds	v
Preening	Uses bill to manipulate feathers on their own body	pr
Allopreening	Uses bill to manipulate feathers on another bird's body	apr
Mutual allopreening	As above but both birds doing this simultaneously	mpr
Pseudo-regurgitation	Bird attempts to regurgitates food but it does not make it to the front of the beak (Bird goes through motion of regurgitating but no food is brought up.	pre
Regurgitation	Bird regurgitates food but does not feed it to the other bird	re

Behavior	Definition	Code
Active- Non-Aggressive (cont.)		
Offers/accepts	Bird offers food outside the nest which is accepted	oa
Offers/accepts in nest	Bird offers food inside the nest which is accepted.	oan
Offers/rejects	Bird offers food outside the next which is rejected	or
Offers/ rejects in nest	Bird offers food inside the nest which is accepted.	orn
Billing	Birds interlock bills without exchanging food	bi
Billing with food	Birds interlock bills and exchange food	bif
Approach	One bird moves within 1m of the other.	ap
Withdraw	Bird moves away with 5 sec of approach by the other bird	w
Out of view	Bird(s) not able to observed because they are hidden from view	oov
Other	Any other activity not covered above	o
Active- Aggressive Behaviors		
Bite	One bird uses its bill to grab another bird (except neck)	b
Stab	Uses tip of bill to strike an object in a fast motion	sta
Neck Bite	Bird pecks or bites at the neck of the other bird	nb
Nudge	Other bird pushes the other with its bill	nu

Nest investigation	Bird extends its head into nest	ni	**Pacing**	Moving back and forth repeatedly in an agitated state	p

Appendix B- URSC Project Budget Justification

URSC PROJECT BUDGET JUSTIFICATION TABLE

Name(s): Taylor Readyhough
Project Title: Great Indian Hornbill Observation

Items *(Please itemize amounts below)*	Description	Funds requested from URSC	Funds requested from other sources	Source of other funds
Supplies				
ZooMonitor Subscription	IPAD BASED APP.	$50	0	X
iPad	FOR USE WITH ZOOMONITOR APP.	0	0	ON LOAN FROM DENVER ZOO
Gasoline	REGULAR UNLEADED GASOLINE	$72.03	0	X
Folding Stool	FROM AMAZON.COM	$19.95	0	X
Total URSC Request		$141.98		

Faculty Advisor name:

Faculty Advisor signature and date:

URSC PROJECT BUDGET JUSTIFICATION NARRATIVE

Please describe **each** item you listed in the budget table. The description should enable reviewers to understand a) how the cost of each item was computed, and b) how the budget items relate to your project objectives.

Supplies:
ZooMonitor Subscription- $50/yr individual subscription- iPad based app. used to collect behavioral data

iPad- $0- On loan from Denver Zoo to use with ZooMonitor app.

Gasoline- $2.49/gal x 28.93 gal= $72.03 (28.93 gal= 14.2 mi round trip x 55 trips x 27 mpg)

Folding Stool- $19.95 from Amazon.com- provides a place to sit during behavioral observations in an exhibit that does not provide a seating area

Research Assistant(s):

Other:

Total Amount Requested from URSC: $____141.98________________________________

CHAPTER 3. JOURNAL MANUSCRIPT: A NOT-SO-SILENT NIGHT: INVESTIGATING THE EFFECTS OF EXTENDED ZOO OPERATING HOURS DURING "ZOO LIGHTS" ON A PAIR OF GREAT INDIAN HORNBILLS

Abstract

Captive animals frequently encounter artificial light, crowds of people, and elevated noise levels compared to natural environments which can act as stressors. Evening events, such as Zoo Lights, increase captive animals' exposure to stressors and may lead to behavioral changes. The pair of great Indian hornbills (*Buceros bicornis*) at the Denver Zoo provide a system to study the impacts of these stressors because their exhibit is open every evening during Zoo Lights. Additionally, the impacts of these stressors may be especially pronounced because Zoo Lights aligns with the beginning of the hornbills' breeding season. I expected the hornbills increase aggressive behaviors during Zoo Lights due to the increased exposure to stressors. Alternatively, I expected the hornbills to engage in more affiliative behaviors, and to increase conspecific and nest proximity, during and after Zoo Lights due to their breeding season. The hornbills engaged in significantly more affiliative behaviors and increased conspecific proximity during *and after* Zoo Lights compared to before. Their behavioral shifts are likely due to their breeding season and not to the increased exposure to stressors during Zoo Lights. The hornbills increased conspecific and nest proximity when crowd size or noise level increased. Additionally, the female was more likely to engage in aggressive behaviors when the noise level was low, whereas the male increased aggressive behaviors when the noise level was medium or high. This indicates that these stressors have acute effects on the hornbills' behaviors despite the lack of overall behavioral changes due to Zoo Lights.

Introduction

In terms of inspiration and education, there is no substitute for someone seeing an elusive wild animal at close proximity (Kruse and Card 2004; Moss and Esson 2010). When most people gain this experience it is, at its core, the opposite of wild. Frequently, this experience is a part of the carefully constructed encounters that play out in zoos around the world. And captivity inherently presents its own challenges by exposing animals to novel experiences and stressors compared to the wild. In captive conditions, animals frequently encounter artificial light, crowds of people, and elevated noise levels compared to natural environments (Collins et al. 2017; Woolway and Goodenough 2017; Larsen et al. 2014; Clark et al. 2012). These factors act as potential stressors for captive animals and can especially impact captive birds (Dickens and Bentley 2014; Owen et al. 2004; Terio et al. 2004). When they are stressed, captive birds damage their feathers, lose interest in novel experiences, and lack exploratory behaviors (Fox 1968; King 1993; Cockrem 2007). Reducing stress levels in captive birds can improve their welfare and encourage successful breeding in the cases when zoos are working to counter the declines in their populations (Conway 2003).

Zoos house endangered exotic birds indoors to control the temperature and humidity levels in their enclosures, and most indoor exhibits use artificial light sources. Light has far-reaching effects: daily light cycles determine the behaviors of birds and many other animals by regulating sleep patterns and reproductive cycles (Turek et al. 1976; Poot et al. 2008; de Jong et al. 2016; Dominoni et al. 2016; Raap et al. 2016; Raap et al. 2017). In captivity, light levels remain crucial to birds' behavior patterns, but the light they experience is not always natural. Elevated levels of artificial light can have profound impacts on captive birds' diurnal activities. Photoperiods, the length of time that animals experience sunlight each day, regulate animals' circadian rhythms throughout their lives (Elliott 1976). Raap et al. (2017) showed that great tits lost approximately 40 minutes of sleep when they were exposed to artificial light inside their nest boxes throughout the night. In another study, the more intense the light was at night, the more active great tits were throughout the day and night (de Jong et al. 2016). Increased exposure to artificial light at night caused decreased nitric oxide levels and increased haptoglobin levels in free-living nestling great tits' blood (Raap et al. 2016). Decreased melatonin levels frequently lead to similar shifts in haptoglobin and nitric oxide levels, so these results may indicate decreased melatonin production in the great tit nestlings. These biochemical shifts represent an increased immune response that consumed valuable energy and resources the nestling birds need to grow (Raap et al. 2016). Continuous exposure to artificial light can dramatically alter captive birds' sleep and activity patterns.

Artificial light also causes adjustments to daily light cycles that alter reproductive development (Dominoni et al. 2016, Elliott 1976). When European blackbirds experienced artificial light at night, their reproductive systems developed almost a month earlier than blackbirds that did not experience artificial light (Dominoni et al. 2016). This shift is important to consider in captive breeding programs because the timing of birds' reproductive development can negatively impact reproductive success (Verhulst and Nilsson 2008). Specifically, birds' clutch sizes and offspring survival decline in the course of a season. This effect seems to be due to a combination of a direct effect of breeding time and an indirect effect due to the quality of the breeding pair (Verhulst and Nilsson 2008). The extended time that captive birds spend in artificially lit environments may have a negative effect on their reproductive cycles and daily activity patterns; therefore, light is an important factor to consider when managing captive birds.

Along with extended exposure to artificial light, captivity forces animals to remain in close proximity to crowds of humans, which can be another stressor for the animals (Morgan and Tromborg 2007). The presence of zoo visitors is frequently a stressful experience for captive animals (Collins et al. 2017; Woolway and Goodenough 2017; Larsen et al. 2014; Clark et al. 2012). For example, citron-crested and Moluccan cockatoos changed their behavior when children were close to the birds' enclosures (Collins and Marples 2015). The citron-crested cockatoos increased their social behaviors, such as allopreening and feeding, while the Moluccan cockatoos retreated from the children (Collins and Marples 2015). This study illustrates the importance of understanding species-specific responses to crowds in captive birds.

Additionally, crowds of people cause increased noise levels that can exacerbate the effects of human presence. Some animals respond more strongly to elevated noise levels than they do to crowd size (Larsen et al. 2014; Davey 2007; Owen et al. 2004). Giant pandas showed an increased hormonal stress response and/or an increased behavioral stress response, in the form of increased locomotion, door manipulation, scratching, and vocalizations, during high levels of anthropogenic noise levels (Owen et al. 2004) and captive koalas spent more time vigilant when ambient anthropogenic noise levels were higher (Larsen et al. 2014). Since increased noise levels are important stressors for many other animals, I would expect a similar response in captive birds.

The captive birds observed in this study were a pair of great Indian hornbills located at the Denver Zoo. Hornbills (Bucerotidae) are an endangered family of birds, distinguished by their large casque, a keratinous structure that sits on top of their hefty bill. In the wild, hornbills reside in Africa and Southeast Asia where their habitat consists of forests, rainforests, and savannahs (Kemp 1993). One of the larger species of the 54 known hornbill species is the great

Indian hornbill, *Buceros bicornis* (Kemp 1993). The great Indian hornbill inhabits China, India, Bhutan, Thailand, Laos, and occasionally a few other Asian countries (Kemp 1993). Great Indian hornbills are sexually dimorphic; females are smaller than males and the males have dark-red eyes whereas the females have light-blue eyes. Great Indian hornbills reside in old growth tropical forests, which are being lost to logging operations (de Ruiter 1998; Sethi & Howe 2009). Consequently, developing successful captive breeding programs is increasingly important in order to support research and conservation efforts for these threatened birds.

The loss of old growth tropical forests is especially impactful for hornbills because hornbills build their nests in cavities that only exist in old growth trees (Poulsen 1970; James & Kannan 2007). Together, the mating pair seals the female into the nest with a mixture of fecal matter and mud for the duration of the incubation period in a process referred to as "mudding in." The pair leaves a small opening in the nest entrance through which the male passes food and the female defecates (Poulsen 1970; James & Kannan 2007). Hornbills are notoriously sensitive breeders because the female must remain in such a vulnerable position for a long period of time (Galama et al. 2002). Therefore, any increases to stress during their breeding season can result in failed reproductive attempts or a lack of interest in mating at all.

The Denver Zoo participates in captive breeding programs for many of their animals, including a pair of great Indian hornbills. However, the pair of great Indian hornbills has never attempted to reproduce, as evidenced by disinterest in the nest box and no attempts to mud-in the female (Vyas pers. comm. 2017). This study focused on the only pair of great Indian hornbills (*Buceros bicornis*) at the Denver Zoo. The months of December – March typically make up the hornbills' breeding season (Vyas pers. comm. 2017), and this study began prior to the breeding season in late October 2017 and continued through the end of February 2018. Additionally, Zoo

Lights, an evening entertainment event that includes large holiday light displays, shows, and access to some of the animal exhibits (Denver Zoo 2017) corresponded with this study period for the entire month of December. The extended hours during Zoo Lights results in increased animal exposure to artificial light, crowds, and noise. In 2017, Zoo Lights occurred daily from 5:30-9:00pm, which equated to approximately four extra hours of exposure to crowds, noise, and artificial light for the animals. The primary goal of this study was to assess whether these extended operating hours had overall negative effects on behaviors of the pair of great Indian hornbills through increased aggressive behaviors during Zoo Lights compared to before and after Zoo Lights.

The great Indian hornbills' zookeepers noticed dramatic behavioral changes during December 2016. They reported that the male was increasingly aggressive towards the female, chasing and biting her so frequently that it was difficult for her to rest in any one place for more than a few minutes. The zookeepers suspected this aggression may have been related to the extended exposure to stressors because the behavioral changes closely matched the timeline of Zoo Lights (Vyas pers. comm.). The impacts from Zoo Lights are exceptionally important during this time of year because of the overlap with the hornbills' breeding season. The increased stress and related aggression may negatively impact the hornbill pair's mating success.

In order to assess the impacts of the extended zoo operating hours during Zoo Lights on the great Indian hornbills, I compared their social behaviors across three time-periods: before, during, and after Zoo Lights. I expected the hornbills to exhibit increased aggressive behaviors due to the extended exposure to stressors during the Zoo Lights time period. Alternatively, if the behavioral changes in the hornbills are due primarily to their breeding season, I expected the hornbills to exhibit increased affiliative behaviors during and after Zoo Lights, and I expected their proximity to each other and the nest box before Zoo Lights to be significantly lower than during and after Zoo Lights. In this case, I expected that their proximity to each other and the nest box during and after Zoo Lights would not be significantly different from each other as both of these time periods encompass the hornbills' breeding season. Additionally, I expected the hornbills to exhibit fewer affiliative behaviors and more aggressive behaviors when crowd size is large and noise level is high due to acute effects from the elevated stressors.

Methods

Study Site

The great Indian hornbills observed in this study reside at the Denver Zoo. Originally, their enclosure was designed for bats but has since been modified to provide a habitat for the hornbills. They are the only species in their enclosure. The hornbills are separated from the public by netting, but there is no solid barrier to reduce noise from the crowd. The exhibit includes several trees with branches and ropes for them to use as perches, however, there is no green, leafy vegetation present (Figure 1). There is a nesting box in one corner of the enclosure and during their mating season (December - March), the zookeepers provide tubs with mud for them to use to "mud-in" the female. They receive natural light through four circular skylights in the roof and there are additional artificial light bars that illuminate the enclosure.

Figure 1: *The central tree in the great Indian hornbill exhibit at the Denver Zoo.*

Data Collection

Due to the condensed time period over which data needed to be collected, I worked with a team of five collaborators to collect data during times that the zoo staff designated as “slow,” “average,” and “busy” regarding visitor attendance each week. We collected data five times per week, for one hour each time. In order to compare the hornbills’ behaviors in relation to Zoo Lights, we gathered data during three periods: before (October/November), during (December), and after Zoo Lights (January/February). We collected 25 hours of observational data during the day in the Pre- and Post- sampling periods, 10 hours during the day in December (during the Zoo Lights time period), and 25 hours during the evening hours of the actual Zoo Lights event (85 hours total). We tested inter-observer reliability using two 10-minute sampling periods with all participants. Our mean inter-observer reliability score was 93% agreement.

We used instantaneous scan sampling (Altmann 1974) to assess the behaviors of both the male and female great Indian hornbills over 1-hour periods, recording behavioral data for both hornbills every minute (Appendix A). In addition to the behavioral data, we also collected 0/1 data for the birds' conspecific proximity (1 = within 1m of each other, 0 = more than 1m from each other) and their nest proximity (1 = within 1m of the nest, 0 = more than 1m from the nest). Crowd and noise data were also collected at each 1-minute interval. Crowd size was recorded as the following categories: small = 1-4 people, medium = 5-8 people, and large = 9+ people. We also used three categories to estimate noise level during the same time intervals: low = most normal background noises are audible, medium = only louder background noises are audible, and high = no normal background noises are audible over the crowd noise.

I coordinated with the zookeepers to minimize the impact of their work on my study. When a zookeeper entered the exhibit, the hornbills' behavior changed drastically because the keepers were associated with food. Therefore, I worked around the keepers' schedules to observe the hornbills when they did not need to enter the enclosure. In the event that the keepers did enter during my collection times, I eliminated the time when the keepers were present plus one minute after they exited the exhibit from my data analysis. This allowed the birds to calm down after the keeper left the exhibit, reducing the potential bias of the keeper interactions.

I worked closely with the Assistant Curator of Birds, Katie Vyas, to determine which behaviors indicated stress. We categorized the hornbills' behaviors into passive, aggressive, and affiliative behaviors (Appendix A- Ethogram). We understood the aggressive category to be indicative of increased stress in the hornbills, while the affiliative category was understood to indicate breeding season behaviors. Behaviors like chasing can be indicative of stress but can also be associated with courtship and mating behaviors (Vyas, pers. comm.). For this study, I considered these behaviors to be aggressive.

Data Analysis

In order to test my hypotheses, I employed various data analysis techniques within the R version 3.4.1 data analysis software (R Core Team 2017). Initially, I tested for correlation between the predictor variables (crowd size and noise level) using a Pearson's Chi-square test. I compared the hornbills' behaviors across my three sampling periods and the different stressor levels with Mantel-Haenszel tests from the stats base package in order to determine if the behaviors were significantly different between sampling periods or between crowd and noise levels (R Core Team 2017). I used the lme4 package to fit binomial generalized linear models (GLMs) to examine changes in nest proximity and conspecific proximity across the three sampling periods (Bates et al. 2015). Then, I used the nnet package to fit multinomial logistic regression models in order to assess if there were significant changes in passive, aggressive, and affiliative behaviors due to increased crowd or noise levels ($\alpha = 0.05$) across all time periods (Venables & Ripley 2002). I also used multinomial logistic regression to assess if there were significant changes in behaviors between the three sampling periods ($\alpha = 0.05$) (Venables & Ripley 2002). All graphs were constructed using the ggplot2 package (Wickham 2009).

Results

Crowd and Noise

Crowd size and noise level were significantly associated with each other ($p < 0.001$, Pearson's Chi-squared Test). Additionally, crowd size ($p < 0.001$, Pearson's Chi-squared Test) and noise level ($p < 0.001$, Pearson's Chi-squared Test) were both significantly associated with the time period. Crowd size was low throughout the during Zoo Lights time period due to the area in front of the exhibit being blocked off (Table 1). Additionally, noise level was medium or high for a larger proportion of time during Zoo Lights compared to before and after (Table 1).

Table 1: *Proportion of scans that fell within each level for crowd size and noise level across the three time periods*

Variable	Level	Before	During	After
Crowd	Small	0.916	0.978	0.936
	Medium	0.062	0.018	0.047
	Large	0.008	0.000	0.016
	Not Recorded	0.014	0.005	0.001
Noise	Low	0.468	0.530	0.745
	Medium	0.082	0.399	0.212
	High	0.011	0.065	0.041
	Not Recorded	0.439	0.006	0.002

**= significant difference based on 95% CI*

Conspecific Proximity

Conspecific proximity, the amount of time the hornbills spent within 1m of each other, was significantly associated with time period ($M^2 = 938.05$; $p < 0.001$; Mantel-Haenszel Test) after accounting for noise level. After accounting for crowd size, noise level, and artificial light, the pair of hornbills spent significantly less time within 1m of each other before Zoo Lights compared to during ($p < 0.001$; Binomial Generalized Linear Model; Figure 2) and after ($p < 0.001$; Binomial Generalized Linear Model; Figure 2). The amount of time the pair spent near each other during and after Zoo Lights were not significantly different from each other ($p = 0.585$; Binomial Generalized Linear Model). The proportion of scans the hornbills spent within 1m of each other before Zoo Lights was 0.001 (95% CI: <0.001 - 0.009; Binomial GLM) and increased to 0.177 (95% CI: 0.026 - 0.636; Binomial GLM) during Zoo Lights. The proportion then increased to 0.243 (95% CI: 0.034 - 0.743; Binomial GLM) after Zoo Lights.

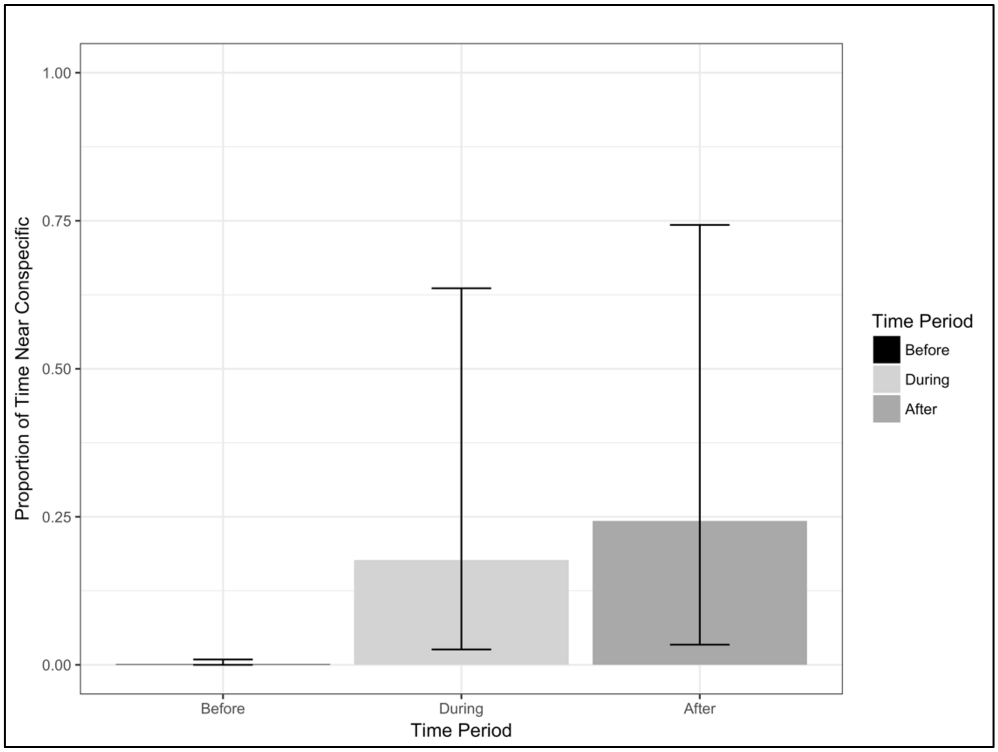

Figure 2: Proportion of time spent near conspecific. *The pair of hornbills spent significantly more time within 1m of each other during and after Zoo Lights in comparison to before Zoo Lights, when all other variables (crowd size, noise level, and light) were accounted for. The amount of time spent within 1m of each other was not significantly different between the during and after time periods.*

Conspecific proximity was also significantly associated with crowd size (p = 0.003; Mantel-Haenszel Test) and noise level (p =0.002; Mantel-Haenszel Test) when time period was accounted for. The hornbills were more likely to be within 1m of each other when crowd size was large than small (p = 0.017, Binomial Generalized Linear Model; Figure 3). After controlling for time period and noise level, the probability that the hornbills were within 1m of each other was 0.003 (95% CI: 0.001 - 0.007; Binomial GLM) when crowd size was small and remained at 0.003 (95% CI: 0.001 – 0.006; Binomial GLM) when crowd size was medium. This probability increased to 0.994 when the crowd size was large (95% CI: 0.986 – 0.998; Binomial

GLM). The probability showed a marginally significant increase from medium to large crowd size (p = 0.093; Binomial GLM).

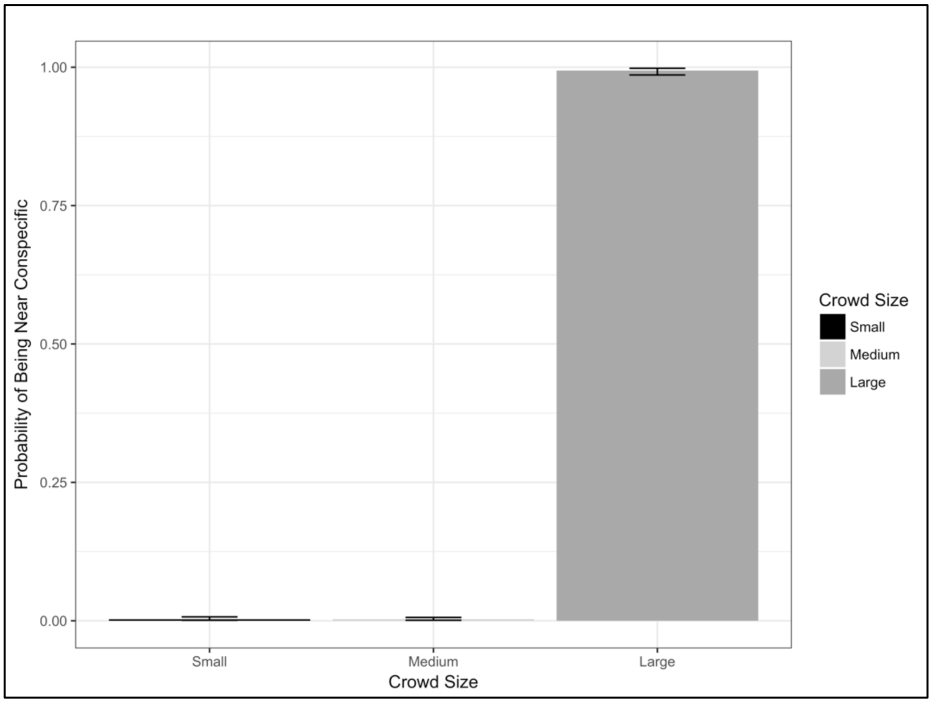

Figure 3: The probability that the hornbills are within 1m of each other in each crowd category. *The pair of hornbills were significantly more likely to be within 1m of each other when crowd size was large.*

The hornbills were also more likely to be within 1m of each other when noise level was high than low (p = 0.024; Binomial Generalized Linear Model; Figure 4). After controlling for time period and crowd size, the probability that the hornbills were within 1m of each other when noise level was low was 0.001 (95% CI: 0.0003 – 0.002; Binomial GLM). This increased to 0.003 (95% CI: 0.001 - 0.006; Binomial GLM) when noise level was medium, and increased again to 0.997 when the noise level was high (95% CI: 0.993 – 0.999; Binomial GLM). This probability showed a marginally significant increase from medium to high noise level (p = 0.080; Binomial GLM).

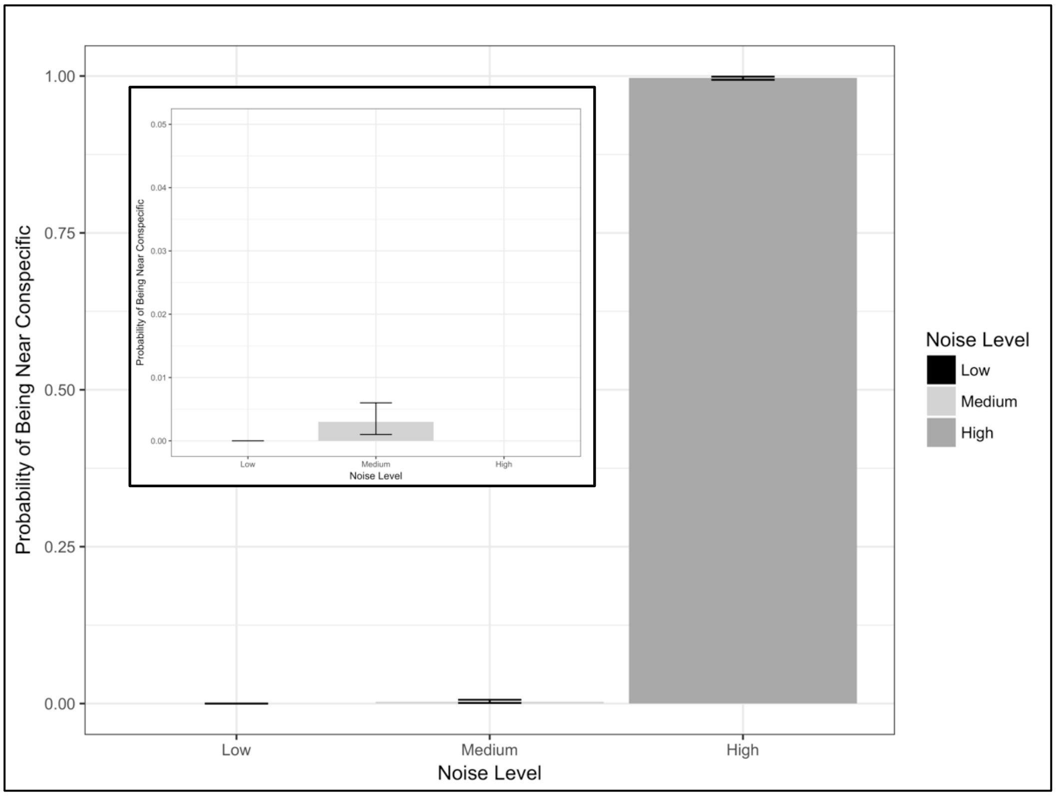

Figure 4: The probability that the hornbills are within 1m of each other during each noise level category. *The hornbills were significantly more likely to be within 1m of each other when the noise level was high.*

Nest Proximity

When crowd size and noise level were held constant, the amount of time both the male ($p < 0.001$; Mantel-Haenszel Test) and female hornbill ($p < 0.001$; Mantel-Haenszel Test) spent within 1m of the nest was significantly associated with the time period. The male and female hornbill only spent time within 1m of the nest after Zoo Lights (Table 2). The female spent less time after Zoo Lights near the nest than the male (Table 2).

Table 2: The Percentage of Scans Spent Near the Nest in each Time Period. *We only observed the male and female hornbill within 1m of the nest after Zoo Lights. The male hornbill spent more time near the nest than the female hornbill after Zoo Lights.*

Individual	**Before**	**During**	**After ***
Female	0.00%	0.00%	12.06%
Male	0.00%	0.00%	16.53%

**= significant difference*

When time period is held constant, the amount of time the female spent within 1m of the nest was not significantly associated with crowd size ($p = 0.180$; Mantel-Haenszel Test) or noise level ($p = 0.527$; Mantel-Haenszel Test). In the same manner, the amount of time the male spent within 1m of the nest was not significantly associated with crowd size ($p = 0.474$; Mantel-Haenszel Test) and was only marginally associated with noise level ($p = 0.064$; Mantel-Haenszel Test).

Behavioral Changes

Response to Time Period

I analyzed changes in aggressive and affiliative behaviors (Appendix A- Ethogram) across the three time periods and determined that both the male and female great Indian hornbills' behaviors were significantly associated with time period when crowd and noise were controlled for (female M^2: 27.438 and 20.848, respectively; $p < 0.001$; male M^2: 118.65 and 101.75, respectively; $p < 0.001$; Mantel-Haenszel Tests). The proportion of scans spent engaged in aggressive behaviors were not significantly different between the three time periods for the male or female hornbill. However, both the male and female great Indian hornbill were significantly more likely to be engaged in affiliative behaviors during and after Zoo Lights compared to before Zoo Lights (Multinomial Generalized Linear Model; Figure 5; Figure 6).

The male hornbill also engaged in significantly more affiliative behaviors after Zoo Lights than before or during Zoo Lights (Multinomial Generalized Linear Model). Overall, both hornbills spent the majority of their time engaged in passive behaviors (Multinomial Generalized Linear Model; Table 3).

Table 3: *Proportion of scans (and 95% confidence intervals) the hornbills engaged in each category of behavior during each time-period. The majority of their time was engaged in passive behaviors.*

Individual	Behavior	Before	During	After
Female	Passive	0.995 (0.986-0.999)	0.981 (0.949-0.992)	0.979 (0.958-0.988)
	Aggressive	0.005 (0.001-0.014)	0.012 (0.004-0.041)	0.006 (0.001-0.022)
	Affiliative	<0.001 * (<0.001)	0.007 * (0.005-0.010)	0.015 * (0.011-0.020)
Male	Passive	0.995 (0.987-0.998)	0.988 (0.978-0.992)	0.937 * (0.894-0.958)
	Aggressive	0.005 (0.002-0.013)	0.003 (0.001-0.009)	0.013 (0.004-0.038)
	Affiliative	<0.001 * (<0.001)	0.009 * (0.007-0.013)	0.050 * (0.037-0.068)

**= significant difference based on 95% CI*

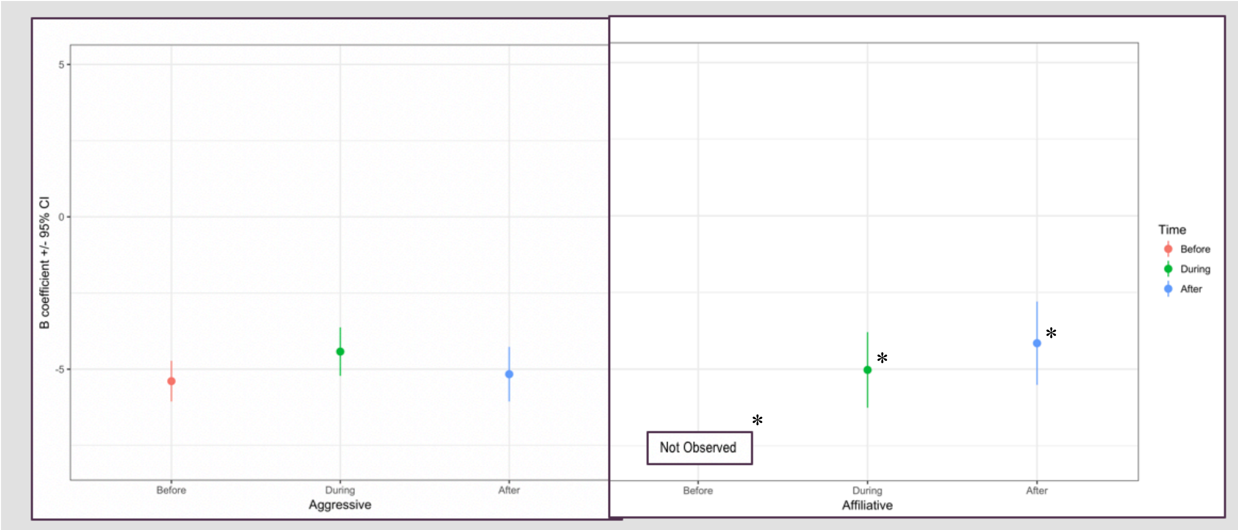

Figure 5: β coefficients of female aggressive and affiliative behaviors compared to passive behaviors. *Comparison of the β coefficients comparing affiliative and aggressive behaviors to passive behaviors (when $\beta_{passive} = 0$). The amount of aggressive behaviors the female engaged in was not significantly different between the three time-periods. Passive behaviors were the most frequently observed behaviors, indicated by the negative β coefficients for aggressive and affiliative behaviors. The female hornbill engaged in significantly more affiliative behaviors during and after Zoo Lights compared to before Zoo Lights. We did not observe any affiliative behaviors before Zoo Lights. The β coefficients for affiliative behaviors are not significantly different from each other during and after Zoo Lights.*

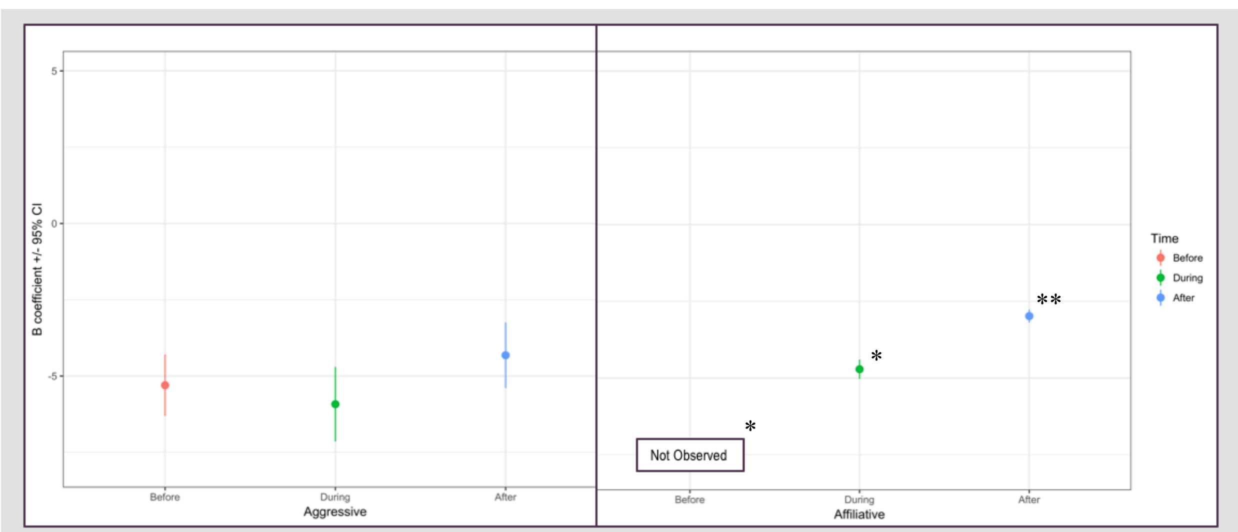

Figure 5: β coefficients of male aggressive and affiliative behaviors compared to passive behaviors. *Comparison of the β coefficients comparing affiliative and aggressive behaviors to passive behaviors (when $\beta_{passive} = 0$). The amount of aggressive behaviors vs. passive behaviors the male engaged in was not significantly different between the three time-periods. Passive behaviors were the most common, indicated by the negative β coefficients for aggressive and affiliative behaviors. The male hornbill engaged in significantly more affiliative behaviors after Zoo Lights compared to before and during Zoo Lights, and significantly more affiliative behaviors during Zoo Lights compared to before Zoo Lights.*

Response to Noise Level

I analyzed changes in aggressive and affiliative behaviors (Appendix A- Ethogram) across the three noise levels (low, medium, and high) and determined that both the male and female great Indian hornbills' behaviors were significantly associated with noise level when crowd and time period were controlled for (female M^2: 20.329; $p < 0.001$; male M^2: 16.936; $p = 0.002$; Mantel-Haenszel Tests). Overall, both hornbills spent the majority of their time engaged in passive behaviors (Multinomial Generalized Linear Model; Table 4). The female great Indian hornbill was more likely to be engaged in aggressive behaviors when noise level was low (Multinomial Generalized Linear Model; Table 4), while the male was more likely to engage in aggressive behaviors when the noise level was medium or high.

Table 4: *Proportion of scans (and 95% confidence intervals) for which the hornbills engaged in each category of behavior during each noise level. The majority of their time was engaged in passive behaviors.*

Individual	Behavior	Low	Medium	High
Female	**Passive**	0.995 (0.986-0.999)	0.998 (0.994-0.999)	0.998 (0.967-0.997)
	Aggressive	0.005 (0.001-0.014)	0.002 (0.001-0.006)	0.002 (0.004-0.033)
	Affiliative	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)
Male	**Passive**	0.995 (0.986-0.999)	0.990 (0.979-0.95)	0.986 (0.951-0.996)
	Aggressive	0.005 (0.001-0.014)	0.010 (0.004-0.021)	0.014 (0.004-0.049)
	Affiliative	<0.001 (<0.001)	<0.001 (<0.001)	<0.001 (<0.001)

**= significant difference from other noise levels*

Discussion

The individual great Indian hornbills responded to Zoo Lights; both the male and female increased their affiliative behaviors during and after Zoo Lights in comparison to before Zoo Lights. Additionally, the pair of hornbills increased the amount of time they spent within 1m of each other and/or the nest during and after Zoo Lights. This behavioral shift implies that the behavioral changes we observed in this pair of hornbills are primarily due to the onset of their breeding season, and not to the stress from the Zoo Lights event. Since Zoo Lights coincides with the start of the great Indian hornbills' breeding season, it is important to assess whether behavioral shifts are consistent throughout their breeding season, or whether these behavioral shifts only occur during Zoo Lights and may be due to the increased exposure to stressors. Examining the impacts of these three stressors during a critical time in the great Indian hornbills' life-history, their breeding season, allows us to understand potential reasons for the lack of breeding success with this pair (Vyas, pers. comm.). Continuing observations throughout their breeding season would add valuable insights into the hornbills' behavioral patterns.

Along with the changes in proximity to each other and the nest during and after Zoo Lights, I also observed effects of crowd size and noise level on conspecific and nest proximity after controlling for the time period in relation to Zoo Lights. Specifically, the hornbills were more likely to be near each other and/or the nest when crowd size was large or noise level was high. Additionally, the female was more likely to engage in aggressive behaviors when noise level was low whereas the male was more likely to engage in aggressive behaviors when noise level was medium or high. These shifts indicate that two of the stressors that the hornbills experience in captivity do have significant acute effects on their social behaviors separately from Zoo Lights.

Unexpectedly, we did not find a significant difference in the percentage of time the hornbills engaged in aggressive behaviors between the time periods. This indicates that the increased exposure to potential stressors does not significantly increase the hornbills' aggressive behaviors. This finding conflicts with the anecdotal evidence from the zookeepers about the hornbills' behaviors in 2016, as well as numerous other studies that have found significant relationships between crowd size, noise level, artificial light, and animals' behaviors (Elliott 1976; Owen et al. 2004; Davey 2007; Larsen et al. 2014; Collins and Marples 2015; de Jong et al. 2016; Raap et al. 2017). It is important to note that the Denver Zoo adjusted the set up during Zoo Lights in 2017 so that crowds of people were not allowed in the area facing the hornbills' exhibit. This led to the hornbills experiencing increased noise levels and more time in the exhibit with artificial lights during Zoo Lights, but no changes in crowd size during Zoo Lights. This change may dampen the effect of Zoo Lights on the hornbills' behavioral responses, but since I controlled for crowd size in my statistical models, I do not expect it to significantly affect the results of this study.

Increased anthropogenic noise has been shown to lead to behaviors that are indicative of stress and to increased aggression in captive animals (Larsen et al. 2014; Davey 2007; Owen et al. 2004). This study supports these previous findings and extends them to avian species because the male great Indian hornbill increased his aggressive behaviors when the noise level was medium and high. However, the female hornbill exhibited the opposite effect, and increased her aggressive behaviors when the noise level was low. This may be due to individual behavioral responses and the female may decrease her interactions under stress, or to the fact that most of my scans occurred when the noise level was low. Additionally, the hornbills were more likely to be within 1m of each other and/or the nest when the noise level was high or the crowd size was large. This is likely due to the collaboration necessary to mud-in the female and the male's role defending the nest once she is mudded inside (Moreau & Moreau 1941; Poulsen 1970). While this pair of hornbills did not mud in the female, investigation is done by both the male and female in a pair prior to selecting a tree cavity for nesting. Additionally, the male defends the nest, and the encapsulated female, from other males and certain predators for the duration of incubation (Moreau & Moreau 1941; Poulsen 1970). It is possible that the male begins defending the female before she is mudded in, and the elevated stressor levels during high noise and large crowd situations stimulate this defense instinct. However, it is important to note that the majority of my scans occurred during low crowd and noise levels (Table 1), which may dampen the significance of crowd size and noise levels as predictors for the hornbills' behaviors and proximity. Further studies could observe the hornbills at random times throughout the day in order to assess the amount of time each day that the hornbills experience high noise and large crowd levels.

Artificial light did not prove to be a significant predictor of the hornbills' behaviors or proximity to each other or the nest in my study. This is most likely due to the fact that most scans (%) occurred when the artificial lights were turned on. However, unlike many previous studies on other birds (de Jong et al. 2016; Raap et al. 2016; Raap et al. 2017), this study did not directly measure the chronic effects of artificial light exposure on the behaviors or reproductive success of the great Indian hornbills. Future studies could investigate the chronic impacts of artificial light exposure on hornbills in order to improve captive breeding success.

The hornbills were not observed within 1m of the nest before or during Zoo Lights. This may be indicative of an increased interest in mating after Zoo Lights, potentially due to the progression of their breeding season or the reduction of excess stressors. While our study ended before the completion of the hornbills' breeding season, future studies should observe the hornbills for the duration of their breeding season in order to examine potential escalation of breeding behaviors over time. Increased breeding behaviors have been found previously in California gulls as they age (Pugesek 1981), and two species of flycatchers vary their foraging behaviors based on the stage of their breeding season (Sakai & Noon 1990). The age of the individuals and the stage of their breeding season may have profound impacts on breeding behaviors and a similar case may exist here with the great Indian hornbills. It would be beneficial to compare a breeding pair exposed to elevated stressors with a breeding pair that does not experience the increased stressor exposure.

The pair of great Indian hornbills did not show increased aggressive behaviors during Zoo Lights, but they did engage in increased affiliative behaviors and spent more time near each other and the nest box during their breeding season (which corresponded with the during and after Zoo Lights time periods). However, even when the hornbills increased their affiliative behaviors, these behaviors occurred during less than 20% of the scans. While their affiliative behaviors increased, the degree to which they increased may not have been enough to reach a threshold for the amount of affiliative behaviors that is indicative of breeding interest. However, my literature search did not yield any studies on breeding behavioral thresholds in avian species. It is likely that such thresholds exist but have not been quantified. Additionally, it is important to understand species-specific differences in behavioral responses to elevated exposure to stressors in order to reduce the negative impact that captivity can have on animals. In this case, the pair of hornbills did not significantly alter their behavior in response to increased time experiencing stressors, which may be indicative of individual adaptation to their captive environment, or a prioritization of breeding behaviors over stress responses. However, they did alter their behaviors in response to increased stressors (such as large crowds and high noise levels) separate from Zoo Lights. This indicates that these stressors have acute effects on the hornbills' social behaviors, but the increased time that the hornbills were exposed to these stressors during Zoo Lights does not seem to have any overarching, chronic effect on their behaviors. The frequency of affiliative behaviors that we did observe may have been lower due to the stressors of Zoo Lights than if the hornbills did not experience the increased stressors from Zoo Lights, and future studies should compare a pair of hornbills exposed to Zoo Lights stressors with a pair that does not experience the increased stressors.

Anecdotally, the hornbills did engage in aggressive behaviors near the nest box. On multiple occasions, I observed the hornbills stabbing at the nest box with their beaks. The female would be inside the nest box stabbing at the corner from within while the male sat on the perch outside and stabbed at the opening to the nest box. The female vocalized in a high pitched, squeaking manner that was very different from the hornbills' normal calls. This would go on for up to five minutes. These behavioral patterns represented an interesting subset of behaviors that only occurred near the nest in the time period after Zoo Lights. My research did not yield any existing descriptions of similar behaviors in any species of hornbills indicating that this behavior has not been extensively studied and is likely rare.

Since artificial light, elevated noise levels, and crowds of people act as stressors for captive animals (Collins et al. 2017; Woolway and Goodenough 2017; Larsen et al. 2014; Clark et al. 2012), it is crucial to expand our understanding of the impacts these environmental variables may have on the behaviors of specific animals. While Zoo Lights did not have a significant impact on the hornbills' behaviors this year, this result may be due to specific changes in the Zoo Lights protocol that were implemented to reduce stress for this pair of hornbills. I do not have data in which crowds of people were allowed near the exhibit during Zoo Lights, so this study was unable to quantify the success of this change. However, numerous other animals experience the same increased stressors during Zoo Lights, which may have profound impacts on their behaviors as well. Expanding behavioral observations to include numerous other species that experience the same stressors would assist the Denver Zoo in assessing the overall impacts that Zoo Lights has on their animal collection and could provide vital information for Zoo policies going forward.

References

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3), 227-266.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software, 67*(1), 1-48. doi:10.18637/jss.v067.i01.

Clark, F. E., Fitzpatrick, M., Hartley, A., King, A. J., Lee, T., Routh, A., ... & George, K. (2012). Relationship between behavior, adrenal activity, and environment in zoo-housed western lowland gorillas (Gorilla gorilla gorilla). *Zoo Biology*, *31*(3), 306-321.

Cockrem, J. F. (2007). Stress, corticosterone responses and avian personalities. *Journal of Ornithology*, *148*(2), 169-178.

Collins, C., Corkery, I., Haigh, A., McKeown, S., Quirke, T., & O'Riordan, R. (2017). The effects of environmental and visitor variables on the behavior of free-ranging ring-tailed lemurs (Lemur catta) in captivity. *Zoo Biology*, *36*(4), 250-260.

Collins, C. K., & Marples, N. M. (2015). Zoo playgrounds: a source of enrichment or stress for a group of nearby cockatoos? A case study. *Journal of Applied Animal Welfare Science*, *18*(4), 375-387.

Conway, W. (2003). The role of zoos in the 21st century. *International Zoo Yearbook*, *38*(1), 7-13.

Davey, G. (2007). Visitors' effects on the welfare of animals in the zoo: A review. *Journal of Applied Animal Welfare Science*, *10*(2), 169-183.

de Jong, M., Jeninga, L., Ouyang, J. Q., van Oers, K., Spoelstra, K., & Visser, M. E. (2016). Dose-dependent responses of avian daily rhythms to artificial light at night. *Physiology & Behavior*, *155*, 172-179.

de Ruiter, M.(1998). The great Indian hornbill a breeding attempt. *AFA Watchbird*, 25(1), 34-35.

Denver Zoo. (2017). *Zoo Lights 2017*. Retrieved from https://www.denverzoo.org/zoolights2017

Dickens, M. J., & Bentley, G. E. (2014). Stress, captivity, and reproduction in a wild bird species. *Hormones and Behavior*, *66*(4), 685-693.

Dominoni, D., Quetting, M., & Partecke, J. (2013). Artificial light at night advances avian reproductive physiology. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1756), e20123017.

Elliott, J. A. (1976). Circadian rhythms and photoperiodic time measurement in mammals. *Federation Proceedings, 35*(12), 2339-2346.

Fox, M. W. (1968). *Abnormal behavior in animals.* Philadelphia: Saunders (W.B.) Co. Ltd.

Galama, W., King, C., & Brouwer, K. (2002). EAZA hornbill management and husbandry guidelines. *Artis Zoo, The Netherlands: National Foundation for Research in Zoological Gardens*.

James, D. A., & Kannan, R. (2007). Wild great hornbills (*Buceros bicornis*) do not use mud to seal nest cavities. *Wilson Journal of Ornithology*, *119*(1), 118-121.

Kemp, A.C. (1993) Conservation of Asian hornbills and their habitats: an introduction, *Manual to the Conservation of Asian Hornbills* (Editors: P. Poonswad and A.C. Kemp), Hornbill Project, Mahidol University Bangkok, pp. 4-23.

King, C. E. (1993). Environmental enrichment: is it for the birds? *Zoo Biology*, *12*(6), 509-512.

Kruse, C. K., & Card, J. A. (2004). Effects of a conservation education camp program on campers' self-reported knowledge, attitude, and behavior. *The Journal of Environmental Education*, *35*(4), 33-45.

Larsen, M. J., Sherwen, S. L., & Rault, J. L. (2014). Number of nearby visitors and noise level affect vigilance in captive koalas. *Applied Animal Behaviour Science*, *154*, 76-82.

Moreau, R. E., & Moreau, W. M. (1941). Breeding biology of Silvery-cheeked Hornbill. *The Auk*, 13-27.

Morgan, K. N., & Tromborg, C. T. (2007). Sources of stress in captivity. *Applied Animal Behaviour Science*, *102*(3), 262-302.

Moss, A., & Esson, M. (2010). Visitor interest in zoo animals and the implications for collection planning and zoo education programmes. *Zoo Biology*, *29*(6), 715-731.

Owen, M. A., Swaisgood, R. R., Czekala, N. M., Steinman, K., & Lindburg, D. G. (2004). Monitoring stress in captive giant pandas (Ailuropoda melanoleuca): behavioral and hormonal responses to ambient noise. *Zoo Biology*, *23*(2), 147-164

Poot, H., Ens, B., de Vries, H., Donners, M., Wernand, M., & Marquenie, J. (2008). Green light for nocturnally migrating birds. *Ecology and Society*, *13*(2).

Poulsen, H. (1970). Nesting behaviour of the black-casqued hornbill *Ceratogymna atrata* (Temm.) and the great hornbill *Buceros bicornis. Ornis Scandinavica*, *1*(1), 11-15

Pugesek, B. H. (1981). Increased reproductive effort with age in the California gull (Larus californicus). *Science*, *212*(4496), 822-823.

R version 3.4.1. R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Raap, T., Casasole, G., Pinxten, R., & Eens, M. (2016). Early life exposure to artificial light at night affects the physiological condition: An experimental study on the ecophysiology of free-living nestling songbirds. *Environmental Pollution*, *218*, 909-914.

Raap, T., Sun, J., Pinxten, R., & Eens, M. (2017). Disruptive effects of light pollution on sleep in free-living birds: season and/or light intensity-dependent? *Behavioural Processes, 144,* 13-19.

Sakai, H. F., & Noon, B. R. (1990). Variation in the foraging behaviors of two flycatchers: associations with stage of the breeding cycle. *Studies in Avian Biology*, *13*, 237-244.

Sethi, P. I. A., & Howe, H. F. (2009). Recruitment of Hornbill-Dispersed Trees in Hunted and Logged Forests of the Indian Eastern Himalaya. *Conservation Biology, 23*(3), 710-718.

Terio, K. A., 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.

Turek, F. W., McMillan, J. P., & Menaker, M. (1976). Melatonin: effects on the circadian locomotor rhythm of sparrows. *Science*, *194*(4272), 1441-1443.

Venables, W. N. & Ripley, B. D. (2002) *Modern Applied Statistics with S*. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Verhulst, S., & Nilsson, J. Å. (2008). The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *363*(1490), 399-410.

Wickham, H. (2009) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Woolway, E. E., & Goodenough, A. E. (2017). Effects of visitor numbers on captive European red squirrels (Sciurus vulgaris) and impacts on visitor experience. *Zoo Biology*, *36*(2), 112-119.

Appendix A- Hornbill Ethogram

Behavior	Definition	Code
Passive (Resting) Behaviors		
Resting	Sitting upright on a perch with no movement of body; small head movements may be noted	r
Vigilant	Watching the surroundings with interest	v
Passive Behaviors		
Stretching	Body is stationary but with significant head/wing/limb movements	str
Eating	Placing bill in food bowl to retrieve items and ingesting those items	e
Flying	Using wings to move from one location to another	f
Hopping	Moving along the length of a perch in a hopping motion	h
Object manipulation	Using bill to make contact with an inanimate object	om
Bill rub	Rubs either side of bill along a perch in a sweeping motion	br
Vocalize	Any vocalization from birds	v
Preening	Uses bill to manipulate feathers on their own body	pr
Out of view	Bird(s) not able to observed because they are hidden from view	oov
Other	Any other activity not covered above	o
Pseudo-regurgitation	Bird attempts to regurgitates food but it does not make it to the front of the beak (Bird goes through motion of regurgitating but no food is brought up.	pre
Regurgitation	Bird regurgitates food but does not feed it to the other bird	re

Behavior	Definition	Code
Affiliative Behaviors		
Offers/accepts	Bird offers food outside the nest which is accepted	oa
Offers/accepts in nest	Bird offers food inside the nest which is accepted.	oan
Offers/rejects	Bird offers food outside the next which is rejected	or
Offers/ rejects in nest	Bird offers food inside the nest which is accepted.	orn
Billing	Birds interlock bills without exchanging food	bi
Billing with food	Birds interlock bills and exchange food	bif
Approach	One bird moves within 1m of the other.	ap
Allopreening	Uses bill to manipulate feathers on another bird's body	apr
Mutual allopreening	As above but both birds doing this simultaneously	mpr
Nest investigation	Bird extends its head into nest	ni
Aggressive Behaviors		
Bite	One bird uses its bill to grab another bird (except neck)	b
Stab	Uses tip of bill to strike an object in a fast motion	sta
Neck Bite	Bird pecks or bites at the neck of the other bird	nb
Nudge	Other bird pushes the other with its bill	nu
Pacing	Moving back and forth repeatedly in an agitated state	p
Withdraw	Bird moves away with 5 sec	w

CHAPTER 4. ENVIRONMENTAL STAKEHOLDER ANALYSIS: THE RED WOLF (*CANIS RUFUS*) AS A CASE STUDY ON ETHICAL CONSIDERATIONS IN CAPTIVE BREEDING FOR REINTRODUCTION

Background

Human impacts on the natural environment have long been known to dramatically decrease native animal populations, and these impacts are especially prominent in the case of apex predators. Beginning in the 1700s, the prominent idea of manifest destiny included dominance over nature, which included the elimination of apex predators, especially wolves (Hinton et al. 2013). As human presence across North America increased, wolves continued to be demonized and efforts towards their extermination continued for the next 200 years. Red wolves (*Canis rufus*) were first described in written works during the 1700s and identified as a unique species in the 1850s. However, they were not extensively studied until the 1960s when interest began to shift away from exterminating wolves and towards rescuing their dwindling populations (Hinton et al. 2013). When this crucial shift occurred, red wolves were already endangered due to over-hunting and hybridization with coyotes (*Canis latrans*).

Almost immediately after scientists at the United States Fish and Wildlife Service (USFWS) began studying red wolves, they initiated a captive breeding program (U.S. Fish and Wildlife Service 1989). Due to the declining red wolf populations, USFWS chose to remove all existing wild red wolves to place them in a breeding facility. Since red wolves appear so similar to coyotes, USFWS captured all canids in the area (approx. 400 individuals) between 1973-1980 and then narrowed them down to 14 full red wolves to use for captive breeding based on phenology. Red wolves were extirpated completely from the wild in 1980, and the only remaining population existed at the red wolf breeding program. Captive breeding of red wolves was relatively successful: the first captive-born litter of pups was born in 1977 and the captive population currently includes approximately 200 red wolves (Phillips et al. 2003).

After captive breeding proved successful, USFWS began searching for an ideal site to reintroduce red wolves to and in 1984, Alligator River National Wildlife Refuge (ARNWR) was established. In 1987, USFWS released eight captive-born wolves into ARNWR and continued to release more than 60 captive-born wolves over the time period from 1987-1994 (Hinton et al. 2013). This reintroduction was considered a success despite the high mortality rates of the wolves (approx. 58% mortality), however, more than half of the red wolf population today still lives in captivity and has not been reintroduced to the wild (Hinton et al. 2013).

In 1991 USFWS attempted to reintroduce red wolves in the Great Smoky Mountains National Park in order to increase the wild populations of red wolves. Unfortunately, this reintroduction was deemed a failure and ended in 1998 due to the migration of most of the red wolves into neighboring agricultural land and a high prevalence of diseases (USFWS 2016; Jenks and Wayne 1992). Even in successful reintroduction cases, the red wolf still faced numerous ecological issues that included hybridization with coyotes and inbreeding (Jenks and Wayne 1992). Hybridization with coyotes is especially important because continued hybridization between red wolves and coyotes is considered the principle threat to stable red wolf populations in the wild. In order to reduce/avoid continued wolf-coyote hybridization, USFWS chose to sterilize the existing coyote population in ARNWR. These sterile coyotes act as place-holders in the ecosystem until red wolves can fill the same niche, and once the sterile coyotes leave the system there should be a convenient gap in the ecosystem for red wolves (Jenks and Wayne 1992). While this approach has mostly limited wolf-coyote hybridization, it is still possible for hybridization to occur due to the migration of new coyotes into the region, and the amount of time and manpower required to capture and sterilize all coyotes (Jenks and Wayne 1992). Additionally, this management strategy only works in controlled environments such as ARNWR and, therefore, this "successful" reintroduction of red wolves may only be a true success in this highly controlled environment.

Ethical Issues

The USFWS captive breeding program arose from the realization that without further intervention, red wolves would become extinct in the near future due to over-hunting and hybridization with coyotes. This hybridization was predicted to occur due to a lack of accessible red wolf mates and was originally considered to be a symptom of the endangered status of red wolves (Jenks and Wayne 1992). Both male and female coyotes and red wolves continue to mate with each other and this continuation of wolf-coyote hybridization implies that this trend is driven by more than just a lack of access to mates (Jenks and Wayne 1992). Additionally, continued hunting of red wolves is permitted under the Endangered Species Act in multiple circumstances and over-hunting remains a threat to red wolf populations, especially the experimental populations that are deemed non-essential by USFWS (USFWS 2016).

Stakeholders and Their Values

The reintroduction of a species is always highly debated, and the reintroduction of an apex predator is even more divisive (Wildlife Management Institute, Inc. and USFWS 2014). Private landowners are important stakeholders when their properties coincide with the planned reintroduction area. However, in the case of the red wolf reintroduction at ARNWR, private land owners, who primarily use their land for hunting, were not originally highly considered in the reintroduction plan. This lead to a general distrust of the USFWS in the reintroduction area. A ban on hunting all coyotes and wolves in response to multiple wolf shootings began in 2013 and exacerbated the existing distrust in the area. Hunters believe that the wolves prey on game animals, such as rabbits, turkeys, and deer, and reduce the local populations of these animals to a point where the hunters' land is "ruined" (Howard 2015). However, research shows that the populations of these game animals did not significantly decline across the five counties in eastern North Carolina where red wolves are present (Howard 2015). In response to the hunting restrictions in 2013, many local hunters believe that since "…the coyotes or the wolves have no natural predators in North Carolina, and they're smart, crafty animals. Anybody's ever tried to hunt them, they have the advantage to start with and you put these restrictions on the hunting, they're going to run rampant…" (Garcia-Pardo and Hertrick 2015). In 2014, more than half of the private land owners near ARNWR do not support the reintroduction of red wolves in the area despite the extant population (Wildlife Management Institute, Inc. and USFWS). Local land owners who use their land primarily for hunting believe that the presence of red wolves is in direct conflict with their hunting success.

In 2016, representatives from six conservation groups (Animal Welfare Institute, Center for Biological Diversity, Endangered Species Coalition, South Florida Wildlands Association, WildEarth Guardians, and Wildlands Network) filed an emergency petition with the USFWS asking for revisions to the 10(j) Rule for red wolves under the Endangered Species Act (Zuardo et al. 2016). Addressed to Daniel M. Ashe, the Director of the USFWS, and Sally Jewell, Secretary of the US Department of the Interior (DOI), this petition maintained that the USFWS was not fulfilling its obligation to satisfactorily protect the endangered red wolf populations and set forth a series of recommended actions for the USFWS to take with their Red Wolf Recovery

Program. The key complaints and adjustments that the petition put forth included: that "the [USFW] Service is illegally dismantling the once successful Red Wolf Recovery Program, the red wolf must be reintroduced to additional areas, the only remaining red wolves in the wild [which are a part of the experimental population] must be considered "Essential" experimental populations, and the 10(j) Rule for red wolves must be revised to reduce shooting deaths" (Zuardo et al. 2016). These representatives from various conservation groups felt that the USFWS was not adequately protecting the red wolf under the ESA. The involvement of these conservation groups aligns with their interests in maintaining biodiversity and preserving endangered species in nature. However, local land owners do not support the increase in wolf populations due to the threat that wolves are to their livestock and pets. The USFWS must balance the interests of the private land owners with the conservation groups, and in doing so, they rely on the written law to support their decisions.

At this same time as the conservation groups petitioned the USFWS, a body of genetic research emerged and complicated the matter even further (Wilson et al. 2000; Brzeski et al. 2016; vonHoldt et al. 2016). Genetic researchers are interested in developing our understanding of the genetic relationships between species and our understanding of what defines a species. Brzeski et al. (2016) analyzed ancient canid mitochondrial DNA samples and found evidence of either a common ancestor or an ancient hybridization event between red wolves and coyotes. This opened the door for further research into the phylogeny of red wolves. On one side, Bridget M. vonHoldt conducted a study at Princeton University and concluded that complete genome sequencing of wolves from various populations across the US, various populations of coyotes, and domestic dogs, indicated that red wolves' genetics were, in fact, an admixture of gray wolf and coyote genetic material. Furthermore, this admixture was indicative of a relatively recent hybridization event, and not the result of two distinct lineages (vonHoldt et al. 2016). These results illustrated an existing issue with the ESA: under the Endangered Species Act, only distinct species can be listed as endangered, and protections for hybrid species are not clearly supported (vonHoldt et al. 2016).

Paul A. Hohenlohe from the University of Idaho and other researchers contested the conclusions made by vonHoldt et al. (2016). While they agree that the genetic data are indicative of admixture through history, and that the ESA (and other legislation) needs to account for hybridization in nature, Hohenlohe et al. (2017) argued that vonHoldt did not directly test the timing of these hybridization events and, therefore, cannot definitively state that there are not distinct evolutionary histories of red wolves, grey wolves, and coyotes. Essentially, Hohenlohe argued that there is not convincing evidence that coyote-wolf admixture occurred at a recent evolutionary time, and there is still a distinct possibility that this admixture occurred long enough ago that red wolves (and eastern wolves) should be considered distinct species (Hohenlohe et al. 2017). vonHoldt et al. (2017) in turn, countered these arguments and stood by their previous conclusion that there are only two distinct canid populations in North America: the grey wolf and the coyote. Continued genetic research will be essential in continuing to investigate the genetic relationships between North American canids and will provide further insights into the evolutionary history of red wolves (Hohenlohe et al. 2017; vonHoldt et al. 2017).

Recommendations and Conclusions

One of the primary reasons conservationists petitioned the USFWS was the lack of protections offered under the ESA to the experimental populations of red wolves from hunting (Zuardo et al. 2016). However, consideration of red wolves and gray wolves as a singular population across the US would change the population count for gray wolves, and lead to the delisting of gray wolves under the ESA (USFWS 2013). This would reduce the protections offered to all wolves in the US and could dramatically reduce or eliminate some populations of wolves (vonHoldt et al. 2016). The ESA is limited by a narrow definition of “species” or “distinct population segment” that qualifies for protection and cannot currently include admixed populations that fill a similar ecological niche similar to their native relatives (Fitzpatrick et al. 2010; O’Brien and Mayr 1991). As emerging genetic research reveals new relationships between species that were not evident due to purely phenotypic relationships, the species protected under the ESA will continue to shift, and revisions to the ESA are necessary in order to protect these unique populations. The definition of a “distinct population segment” under the ESA requires further refinement in order for agencies to prove that a certain population of hybrids or a distinct sub-species meets this definition. The ESA must adapt to reflect the emerging research on genetic relationships between species and to consider the ecological role a population plays.

Additionally, a decision must be made about the future of the Red Wolf Recovery Program, and the existing red wolf populations. While the evolutionary history of red wolves is still highly debated, I recommend that the USFWS continue the conservation program until such a time that the question of red wolves’ status as hybrids or a distinct species can be fully answered. However, the private land owners in the reintroduction area do not support the presence of red wolves. In order to ensure the safety and continuation of the red wolf population in ARNWR, the USFWS must actively engage the community in understanding the benefits and importance of conserving this imperiled population. The USFWS should increase public outreach and actively address public concerns about the red wolf presence through public hearings and educational campaigns. Additionally, both Hohenlohe et al. and vonHoldt et al.

contend that haplotype analysis would be useful for determining more information about the evolutionary timeline of wolf-coyote hybridization (Hohenlohe et al. 2017; vonHoldt et al. 2017). Therefore, haplotype research should begin as soon as possible in order to assist USFWS in making current management decisions for the red wolves. The red wolf is an example of the complex nature of species interactions in the natural world, and wildlife managers should employ every tool in order to base their decisions on the best available science.

References

Adams, J. R., Kelly, B. T., & Waits, L. P. (2003). Using faecal DNA sampling and GIS to monitor hybridization between red wolves (Canis rufus) and coyotes (Canis latrans). *Molecular Ecology*, *12*(8), 2175-2186.

B. M. Fitzpatrick, J. R. Johnson, D. K. Kump, J. J. Smith, S. R. Voss, H. B. Shaffer, Rapid spread of invasive genes into a threatened native species. Proc. Natl. Acad. Sci. U.S.A. 107, 3606–3610 (2010).

Brzeski, K. E., DeBiasse, M. B., Rabon Jr, D. R., Chamberlain, M. J., & Taylor, S. S. (2016). Mitochondrial DNA variation in southeastern pre-Columbian canids. *Journal of Heredity*, *107*(3), 287-293.

Garcia-Pardo, G. (Producer), & Hertrick, J. (Senior Producer). (2015). *Is it OK to let these wolves go extinct in the wild?* [Motion picture]. United States: National Geographic.

Hinton, J. W., Chamberlain, M. J., & Rabon, D. R. (2013). Red wolf (Canis rufus) recovery: a review with suggestions for future research. *Animals*, *3*(3), 722-744.

Hohenlohe, P. A., Rutledge, L. Y., Waits, L. P., Andrews, K. R., Adams, J. R., Hinton, J. W., ... & White, B. N. (2017). Comment on "Whole-genome sequence analysis shows two endemic species of North American wolf are admixtures of the coyote and gray wolf". *Science Advances*, *3*(6), e1602250.

Howard, B. C. (2015). For world's only wild red wolves, a fateful decision. *National Geographic.* Retrieved from https://news.nationalgeographic.com/2015/03/150318-red-wolves-north-carolina-conservation-reintroduction-science/

Jenks, S. M., & Wayne, R. K. (1992). Problems and policy for species threatened by hybridization: the red wolf as a case study. In *Wildlife 2001: Populations* (pp. 237-251). Springer, Dordrecht.

Kalinowski, S. T., Hedrick, P. W., & Miller, P. S. (1999). No inbreeding depression observed in Mexican and red wolf captive breeding programs. *Conservation biology*, *13*(6), 1371-1377.

Kleiman, D. G. (1989). Reintroduction of captive mammals for conservation. *BioScience*, *39*(3), 152-161.

O'Brien, S. J. , & Mayr, E. Bureaucratic mischief: Recognizing endangered species and sub-species. *Science* 251, 1187–1188 (1991).

Phillips, M. K., Henry, V. G., & Kelly, B. T. (2003). Restoration of the red wolf.

Red Wolf Recovery/Species Survival Plan; U.S. Fish and Wildlife Service: Atlanta, GA, USA, 1989; p. 110.

U.S. Fish and Wildlife Service (2016). Causes of mortality in wild red wolves (*Canis rufus*) 2013- 2016. available at https://www.fws.gov/redwolf/Images/Mortalitytable.pdf.

Wildlife Management Institute, Inc. and U.S. Fish and Wildlife Service (2014). A Comprehensive Review and Evaluation of the Red Wolf (Canus rufus) Recovery Program.

vonHoldt, B. M., Cahill, J. A., Fan, Z., Gronau, I., Robinson, J., ... & Wayne, R. K. (2016). Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. *Science Advances*, *2*(7), e1501714.

vonHoldt, B. M., Cahill, J. A., Gronau, I., Shapiro, B., Wall, J., & Wayne, R. K. (2017). Response to Hohenlohe et al. *Science Advances*, *3*(6), e1701233.

Wayne, R. K., & Gittleman, J. L. (1995). The problematic red wolf. *Scientific American*, *273*(1), 36-39.

Wilson, P. J., Grewal, S., Lawford, I. D., Heal, J. N., Granacki, A. G., Pennock, D., ... & Chambers, R. E. (2000). DNA profiles of the eastern Canadian wolf and the red wolf provide evidence for a common evolutionary history independent of the gray wolf. *Canadian Journal of zoology*, *78*(12), 2156-2166.

Zuardo, T., Adkins, C., Huta, L., Schwartz, M., Cotton, B., & Sutherland, R. (2016). Emergency petition to revise the red wolf's 10(j) rule. https://ecos.fws.gov/docs/petitions/92000/884.pdf