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# Winter Ecology of Sandhill Cranes (*Grus canandensis*) in Northern Mexico

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# WINTER ECOLOGY OF SANDHILL CRANES (*Grus canadensis*) IN NORTHERN MEXICO

by

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# WINTER ECOLOGY OF SANDHILL CRANES (*Grus canadensis*) IN NORTHERN MEXICO

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University of Nebraska, 2013

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Sandhill Cranes (*Grus canadensis*) are a widespread species in North America and one of the most studied cranes in the world. However, most of the research has focused on the breeding grounds of Canada and Alaska and the staging grounds of Nebraska. Although an important proportion of the Mid-continent Population of Sandhill Cranes winters in northern Mexico, little information exists on distribution, status, and ecology of the species in Mexico. The goal of this dissertation was to provide new information on Sandhill Crane winter ecology from a regional perspective to better understand population trends. I examined the physiological state of Sandhill Cranes in wild conditions by quantifying the effects of environmental factors on stress levels. My data suggests that access to water resources is the main factor affecting corticosterone levels of cranes. I validated a method to measure glucocorticoid metabolites in fecal samples using an affordable and commercially available enzyme immunoassay. I demonstrated that the use of an enzyme immunoassay provides accurate measurements of steroid metabolite concentrations comparable to the traditional radioimmunoassay. I examined winter diet of Sandhill Cranes and investigated if the species exhibits a specialized or generalized diet in Mexico. I also explored the ecological response of the

species to low food availability conditions. According to my results, cranes exhibit a specialized diet of corn during winter as patterns of consumption did not vary with corn availability. Cranes responded to low food availability by moving geographically to a location where corn was available instead of shifting diets. Finally, I included a human dimensions perspective to document the attitudes of rural inhabitants towards Sandhill Cranes. I investigated if crop consumption by cranes represented a problem for Mexican farmers in the wintering grounds. The results of my interviews indicate that Mexican farmers are not affected by the arrival of cranes and do not consider the species to be a problem.

*For my grandfather Joan Barceló Cabré*

"Far beyond him the sky suddenly exhibited a rotating circle of white spots, alternatively visible and invisible. A faint bugle note soon told us they were cranes, inspecting their Delta and finding it good. At the time my ornithology was homemade, and I was pleased to think them whooping cranes because they were so white. Doubtless they were sandhill cranes, but it doesn't matter. What matters is that we were sharing our wilderness with the wildest of living fowl. We and they had found a common home in the remote fastnesses of space and time; we were both back in the Pleistocene. Had we been able to, we would have bugled back their greeting. Now, from the far reaches of the years, I see them wheeling still." (Leopold 1949: 148)

Sandhill Crane (*Grus canadensis*), Babícora, Chihuahua, Mexico

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# CHAPTER 1: INTRODUCTION

Migratory birds are experiencing rapid declines worldwide (Morton and Greenberg 1989, Robbins et al. 1989, Terborgh 1989, Askins et al. 1990). One-third of all Nearctic migrant birds that winter in the Neotropics are reporting severe declines (Hagan and Johnston 1992). Events that occur during one portion of the annual cycle of a migratory bird have been suggested as causes of declines (Rappole and McDonald 1994). Some of the threats that migratory species are exposed to include habitat fragmentation of breeding, staging, and wintering grounds due to development, land conversion, and habitat degradation, collisions with buildings and communication towers, poisoning by pesticides, predation by introduced predators, and global climate change (Rappole 1995, Both et al. 2006). Recent research has revealed that conservation efforts undertaken in the breeding grounds are later undermined in the wintering grounds (Townsend et al. 2009). Many of the regular breeders in North America are Nearctic-Neotropical migratory birds that breed in the USA and Canada, and winter in Latin America and the Caribbean. Although important efforts on habitat protection in North America have proven successful for breeding birds (Hart et al. 2010), these efforts do not ensure the survival of migratory species. In fact, previous studies indicate that populations of Nearctic migrants appear to be controlled by events that occur in the wintering grounds (Rappole and McDonald 1994). The alarming declines of migratory birds led to the creation of the Migratory Bird Treaty Act of 1918. The treaty protects migratory birds, their feathers, nests, and eggs and prohibits their trading; however, the treaty does not include habitat protection.

Sandhill Cranes (*Grus canadensis*) are Neotropical migrants that were once in decline during the first half of the 1900s (Tacha et al. 1992). Historic records suggest that the species was more widely distributed before the European settlement which caused the species decline due to overhunting, agricultural expansion, and conversion of wetlands affecting its habitat specially in the south of its range (Tacha et al. 1992). Since then, several conservation measures, including the Migratory Bird Treaty Act and hunting regulation, has helped the species recover and is classified as *Least Concern* by the International Union for Conservation of Nature (IUCN).

Sandhill Cranes are long distance migrants; however, not all of their populations are migratory. There are nine recognized populations of Sandhill Cranes in North America, including the non-migratory Cuba, Florida, and Mississippi Populations, and the migratory Eastern, Rocky Mountain, Central Valley, Colorado River Valley, Pacific Coast, and Mid-continent Populations (Tacha et al. 1992). Among the migratory populations, the length and route of their migration varies greatly. The Mid-continent Population is further divided into two subpopulations, including the Western and the Gulf Coast Subpopulations. Recent research using satellite telemetry revealed that individuals from the Mid-continent Population use different breeding grounds and fall staging locations which allowed a further divide of the population into four breeding affiliations (Krapu et al. 2011). This new information provides evidence about the cranes wintering in Mexico. The Sandhill Cranes included in this dissertation belong to the Western Subpopulation of the Mid-continent Population, to the Western Alaska-Siberia breeding affiliation, and belong to the Lesser Sandhill Crane (*G. c. canadensis*) subspecies, also known as artic-nesting cranes (Krapu et al. 2011). These are the cranes that migrate the

longest distance (i.e., up to 8,000 km) between Russia and Mexico crossing through the Bering Strait. Another two breeding affiliations from the Mid-continent Population also winter in Mexico but in smaller numbers, to the east, in the state of Tamaulipas (Krapu et al. 2011); to the west, individuals from the Colorado River Population winter in the states of Sonora and Sinaloa (Tacha et al. 1992); however, these cranes are not the scope of this dissertation.

The majority of birds (82%) from the Mid-continent Population winter in Texas, followed by Oklahoma, Kansas, New Mexico, and Arizona in the USA. It is estimated that 14% of the population winters in northern Mexico, mainly in the states of Chihuahua and Coahuila (Drewien et al. 1996, Krapu et al. 2011). Sandhill Cranes are considered a threatened species in Mexico and are provided special protection (SEMARNAT 2010). Such classification implies that the species could become endangered in the future due to factors that affect their survivorship and therefore the government determines the existence of a necessity to promote its conservation. Although cranes are protected in Mexico, hunting is allowed in designated areas such as UMAS (Unidades de Manejo para la Conservacion de la Vida Silvestre). Sandhill Cranes are a clear example of a globally abundant species that becomes locally threatened. The Mexican government classified the species as threaten as a response to a reduction in their distribution range. Historically, cranes used to winter as far south as the state of Puebla in central Mexico and as far east as the Yucatán Peninsula (Leopold 1965), with accounts in Baja California, Colima, Jalisco, and Quintana Roo (last seen in 1949; Howell and Webb 1995). Although there has been no research to understand the reasons for the reduction in the distribution of Sandhill Cranes in Mexico, information from local residents and

documented changes in agricultural practices suggest that the species no longer finds suitable habitat to winter in the south of the country. Habitat availability has been identified as the most important limiting factor for Sandhill Crane populations (Cooper 1996). More specifically, degradation of wetland habitat has been recognized as the leading threat to the species (Meine and Archibald 1996).

Most studies on Sandhill Cranes have focused on the breeding grounds of Canada and Alaska and the wintering grounds of Texas; prompting an information gap on crane distribution, status, and general ecology in Mexico. The Cranes Status Survey and Conservation Action Plan identified a lack of information for Sandhill Cranes in the wintering grounds of Mexico and recognized the need for research in this area of its distribution as a tool to ensure conservation of the species (Meine and Archibald 1996). Information gained on the ecology of Sandhill Cranes in Mexico may lend valuable insight into how other grassland and waterbirds will respond to increasing threats to persistence in the region and can therefore serve as a framework for conservation of migratory species in wintering grounds at-large.

This dissertation focuses on Sandhill Crane winter ecology from a regional point of view to better understand population trends and explore ways that future population declines can be mitigated. I begin this document by examining the physiological state of Sandhill Cranes in Mexico and how such state may vary during the winter. The physiological state of a population provides information about its condition and fitness. I quantified the adrenocortical response (i.e., stress response) to environmental conditions that affect Sandhill Cranes. Environmental conditions that can be a source of stress differ by wintering sites selected by cranes in terms of food and water resources availability,

predator abundance, human presence, and crane abundance. Prolonged and acute stress levels can be detrimental because they affect resistance to disease, reproductive output, and even survival having a broader effect by potentially diminishing fitness of the entire population. I tested whether stress levels of cranes vary due to exposure to environmental stress factors (Chapter 2).

I measured the stress response of Sandhill Cranes by quantifying the concentration of glucocorticoid metabolites in their feces. Fecal glucocorticoid assays have proven to be very valuable as a non-invasive method to assess stress hormones in wild populations that are difficult to capture. Corticosterone is the main glucocorticoid in avian plasma and the most accurate measure of stress. Corticosterone concentration can be measured using enzyme immunoassays (EIA) which provides advantages in terms of safety and cost over the traditional radioimmunoassay (RIA). I validated the use of an affordable and commercially available corticosterone kit that uses EIA to measure stress hormones non-invasively in wild Sandhill Cranes (Chapter 3).

I next examine winter diet of Sandhill Cranes in Mexico and compare it with previous food studies. Most diet studies suggest that Sandhill Cranes are opportunistic and omnivorous during most stages of their annual cycle; however, food resources in Mexico may differ from other wintering grounds in southern USA. I investigated if the species exhibits a specialized or a generalized diet while wintering in Mexico by quantifying diet diversity through niche breadth. I also compared diets between sites with different food availability. I quantified diet similarities between sites through niche overlap as the relative utilization of different resource components and the amount of overlap in the use of those components. I explored the ecological response of Sandhill

Cranes wintering in areas with low food availability to determine if they either move to a new location or they change their diet (Chapter 4).

The final research chapter of this dissertation focuses on a human dimensions perspective by exploring the attitudes of rural inhabitants towards Sandhill Cranes in northern Mexico. Crop depredation by Sandhill Cranes is a problem in some breeding and staging areas. The problem varies depending on the area, crop type, and time of the year. I investigated if the species represents a problem in this part of Mexico by examining the perceptions that Mexican farmers have developed towards cranes. I documented the proportion of farmers who consider that cranes represent a major threat to their crops; and I also examined the mitigation tactics that farmers may be using to protect or reduce crop losses (Chapter 5).

**LITERATURE CITED**

Askins, R. A., J. F. Lynch, and R. Greenberg. 1990. Population declines in migratory birds in eastern North America. Current Ornithology 7:1-57.

Both, C., S. Bouwhuis, C. M. Lessells, and M. E. Visser. 2006. Climate change and population declines in a long-distance migratory bird. Nature 441:81-83.

Cooper, J. M. 1996. Status of the Sandhill Crane in British Columbia.*in* M. o. Environment, editor. BC Environment, Victoria, BC.

Drewien, R. C., W. M. Brown, and D. S. Benning. 1996. Distribution and abundace of sandhill cranes in Mexico. Journal of Wildlife Management 60:270-285.

Hagan, J. H., and D. W. Johnston. 1992. Ecology and conservation of neotropical migrant landbirds. Smithsonian Institution Press, Washington, D.C.

Hart, J. A., C. C. Rimmer, R. Dettmers, R. M. Whittam, E. A. McKinnon, and K. P. MacFarland. 2010. A conservation action plan for Bicknell's Thrush (*Catharus bicknelli*). International Bicknell's Thrush Conservation Group.

Howell, S. N. G., and S. Webb. 1995. A guide to the birds of Mexico and northern central America. Oxford University Press, New York.

Krapu, G. L., D. A. Brandt, K. L. Jones, and D. H. Johnson. 2011. Geographic distribution of the Mid-Continent Population of sandhill cranes and related management applications Wildlife Monographs 175:1-38.

Leopold, A. S. 1965. Fauna silvestre de Mexico: Aves y mamiferos de caza. Instituto Mexicono de Recursos Naturales Renovables, Mexico, D.F.

Meine, C. D., and G. W. Archibald. 1996. The cranes: Status survey and conservation action plan. IUCN, Gland, Switzerland, and Cambridge, U.K.

Morton, E. S., and R. Greenberg. 1989. The outlook for migratory songbirds: "Future shock" for birders. American Birds 43:178-183.

Rappole, J. H. 1995. The ecology of migrant birds: a neotropical perspective. Smithsonian Institution Press, Washington, D.C.

Rappole, J. H., and M. V. McDonald. 1994. Cause and effect in population declines of migratory birds. The Auk 111:652-660.

Robbins, C. S., J. R. Sauer, R. Greenberg, and S. Droege. 1989. Population declines in North American birds that migrate to the Neotropics. Proceedings of the National Academy of Sciences 86:7658-7662.

SEMARNAT. 2010. Proteccion ambiental - Especies nativas de Mexico de flora y fauna silvestres - Categorias de riesgo y especificaciones para su inclusion, exclusion o cambio - Lista de especies en riesgo. Secretaria de Medio Ambiente y Recursos Naturales. Diario Oficial de la Federacion NOM-059-SEMARNAT-2010.

Tacha, T. C., S. A. Nesbitt, and P. A. vohs. 1992. Sandhill Crane (*Grus canadensis*). Ithaca: Cornell Lab of Ornithology.

Terborgh, J. W. 1989. Where have all the birds gone? Princeton University Press, Princeton, New Jersey.

Townsend, J. M., C. C. Rimmer, and K. P. McFarland. 2009. Investigating the limiting factors of a rare, vulnerable species: Bicknell's Thrush. Proceedings of the Fourth International Partners in Flight Conference: Tundra to Tropics:91-95.

# CHAPTER 2: FACTORS INFLUENCING STRESS LEVELS OF SANDHILL CRANES WINTERING IN NORTHERN MEXICO

## ABSTRACT

Environmental conditions can be a source of stress in wild animals. However, most avian studies have examined stress levels in controlled laboratory conditions or in animals kept in captivity and through plasma samples. I examined the effects of both environmental and human-induced factors on stress levels of Sandhill Cranes (*Grus canadensis*) in wild conditions. I quantified glucocorticoid metabolites (i.e., stress levels) in fecal samples collected in northern Mexico during the winters of 2007 and 2008 using an enzyme immunoassay (EIA). I collected 331 fecal samples from six wetlands. Mean corticosterone concentration during the study period was 301.3±50.9 ng/g of dry feces. Corticosterone levels varied significantly among sites where Sandhill Cranes were exposed to different environmental stress factors ($P < 0.0001$). Time of sampling played a major role in the measure of stress levels. Corticosterone levels significantly increased from November to February ($P = 0.001$). Access to water resources ($\beta_{WetS} = -0.409$, SE = 0.085, 95% CI: -0.579 to -0.238) was the only factor affecting stress levels. Cranes wintering in small and temporary wetlands had higher corticosterone levels than cranes wintering in large and permanent water bodies. High levels of stress hormones affect individual fitness and can be detrimental for the population of cranes. Wetlands in this region have been heavily decimated for agricultural purposes affecting waterbirds that use them as their wintering grounds. My results suggest that cranes are sensitive to available water in the landscape and provide information about some of the consequences

of wetland desiccation. Wetland conservation efforts can be prioritized with such information in northern Mexico.

## INTRODUCTION

Wild animals experience two main sources of stress, environmental stress and human-induced stress. Due to the potential deleterious effects of chronic stress in animals and its important conservation implications ( Millspaugh and Washburn 2004), several studies have investigated the effects of both environmental and human-induced disturbances in different species of mammals: African Elephant (*Loxodonta africana*; Foley et al. 2001), Elk (*Cervus elaphus*; Millspaugh et al. 2001), White-tailed (*Odocoileus virginianus*; Verme and Doepker 1988) and Mule Deer (*O. hemionus*; Saltz and White 1991), Rocky Mountain Bighorn Sheep (*Ovis canadensis canadensis*; Miller et al. 1991), African Wild Dog (*Lycaon pictus*; Creel et al. 1997); reptiles: Fence Lizard (*Sceloporus occidentalis*; Dunlap and Schall 1995); and birds: California (*Strix occidentalis occidentalis*; Tempel and Gutierrez 2003) and Mexican Spotted Owl (*S. o. lucida*; Delaney et al. 1999), Western Marsh Harrier (*Circus aeruginosus*; Fernandez and Azkona 1993), Red-tailed (*Buteo jamaicensis*; Andersen et al. 1989) and Ferruginous Hawk (*B. regalis*; White and Thurow 1985), Bald Eagle (*Haliaeetus leucocephalus*; Grubb and King 1991), and gulls (Kanwisher et al. 1978). Birds that are exposed to stress have been known to react in different ways including flush responses (Andersen et al. 1989, Grubb and King 1991, Stalmaster and Kaiser 1997), home-range shifts (Andersen et al. 1990), reduced parental care of nestlings (Fernandez and Azkona 1993), and reduced reproductive output (White and Thurow 1985).

Mammals and birds have a pair of adrenal glands that regulate the stress response through the synthesis of corticosteroids and catecholamines, including cortisol and adrenaline (Ringer 1976). Cortisol is the major adrenal steroid in medium to large mammals and corticosterone is the major adrenal steroid in avian and small mammal's plasma (Hadley 1996). Animal physiological changes are indicative of a stress response and initiated through the secretion of such hormones (Wingfield and Ramenofsky 1999). These stress hormones are released when the hypothalamic-pituitary-adrenal (HPA) axis is activated (Harvey et al. 1984). The HPA axis, along with other neuroendocrine axes, supports physiologic, morphologic, and behavioral adjustments in response to environmental stimuli (Carsia and Harvey 2000). A stress response develops when these stimuli reach sufficient intensity and/or duration to threaten homeostasis (i.e., constant physiological conditions). Food deprivation, weather extremes, predator recognition, overcrowding, muscular exertion, capture, restraint, and blood collection are examples of stressors that have been correlated with elevations of corticosterone in several species of birds (Harvey and Hall 1990, Le Maho et al. 1992, Siegel 1995, Silverin 1998, Cockrem and Silverin 2002, Quillfeldt and Mostl 2003, Thaker et al. 2009).

Chronic activation of the stress response creates a state of ongoing physiological arousal. This occurs when an animal experiences so many stressors that the autonomic nervous system rarely has a chance to activate the relaxation response. Animals are built to handle acute stress but not chronic stress (Ladewig 2000) and prolonged stress may affect resistance to disease, reproductive output, and even survival (Verme and Doepker 1988, Wingfield 1988, Dunlap and Schall 1995, Foley et al. 2001). In addition, the fight-or-flight response which is meant to help fight a few life-threatening situations spaced out

over a long period, if activated continually can weaken the immunologic system (Moberg 2000).

Quantifying the level of stress that free-ranging birds are exposed to is challenging. However, several techniques have been developed to measure animal stress responses (i.e., adrenocortical activity). These techniques can be divided into invasive and non-invasive techniques that cover both behavioral and physiological responses. Among the invasive techniques are physiological measurements such as plasma parameters and heart-rate monitoring (Kanwisher et al. 1978, MacArthur et al. 1982, Moen et al. 1982). Both of these methods are intrusive because they require handling the animal and we now know that unless the sample is collected between a certain amount of time, capturing and restraining an animal has an effect on stress level measurements (Le Maho et al. 1992, Siegel 1995). Among the non-invasive techniques are behavioral indicators (White and Thurow 1985, Andersen et al. 1989, Andersen et al. 1990, Grubb and King 1991, Fernandez and Azkona 1993, Stalmaster and Kaiser 1997, Rushen 2000) and physiological measurements such as fecal glucocorticoids (Miller et al. 1991, Wingfield et al. 1994a, Creel et al. 1997, Wasser et al. 1997, Wasser et al. 2000, Baltic et al. 2005) and urinary cortisol (Delguidice et al. 1989, Miller et al. 1991, Saltz and White 1991).

The advantages of using fecal glucocorticoid analyses to measure stress hormone secretion is that it allows the quantification of environmental disturbances much earlier in the event than a behavioral method (Foley et al. 2001), giving the chance to find mitigation solutions before it is too late to contain the damage. Another advantage of the fecal glucocorticoid analyses is that the samples are easy to collect year round and each

sample provides an integrated measure of all corticosteroid secretion during the previous 1 to 2 days (Harper and Austad 2000). In contrast, plasma samples are known as "point samples" because they indicate the hormonal status of an individual at a certain point in time (Goymann 2005). Excreta samples contain the pooled amount of excreted hormones allocated to one dropping and for this reason they represent an integral measure of hormone metabolites (Goymann 2005). Although the actual hormone is measured in plasma samples, the circulating hormone itself is no longer present in excreta samples and therefore, the metabolites of the original hormone are measured (Goymann et al. 2002a, b, Palme et al. 2005).

Stress assessments through fecal corticoid concentrations have been performed with Whooping Cranes (*Grus americana*) undergoing reintroduction (Hartup et al. 2005), Greater Sandhill Cranes (*G. canadensis tabida*) being trained to migrate (Hartup et al. 2004), and Florida Sandhill Cranes (*G. c. pratensis*) in captivity (Ludders et al. 2001), but there are no studies of fecal corticoid levels of any species of crane in wild conditions. In fact, very few studies have explored the effects of environmental conditions on stress in wild birds.

It is estimated that approximately 14% of the Mid-continent Population of Sandhill Crane winters in northern Mexico (Drewien et al. 1996, Krapu et al. 2011) in the Chihuahuan Desert Ecoregion with a mean annual rainfall between 125 and 400 mm (Ferrusquia-Villafranca et al. 2005). The region is characterized by a series of basins and ranges with a central highland dominated by shrub communities. The only major flow of freshwater is provided by the Río Grande, fed by its major tributaries the Río Pecos and the Río Conchos (Dinerstein et al. 2001). However, most of the water bodies of this

region originate due to the presence of endorheic basins that are fed by rainfall or small streams and where the topography prevents the drainage to any other water system, not even through underground drainage (Carrera and de la Fuente 2003). These closed basins are characteristic of desert environments where water is only lost through evaporation creating a high concentration of minerals and often becoming saline lakes. Seasonal streams and springs in these isolated basins give way to a network of shallow wetlands spread throughout the region that attract wintering Sandhill Cranes. Wetlands represent critical habitat for cranes; cranes use wetlands as roost sites during the night and rest sites during the hottest hours of the day. Although the Chihuahuan Desert hosts a unique set of biological communities and specialized habitats (Dinerstein et al. 2001) it is considered a low productivity ecosystem (Stoleson et al. 2005) that somehow provides enough food resources to sustain a wintering population of Sandhill Cranes. The combination of water unpredictability, a characteristic of arid environments (Bauder 2005), and low food production suggests that Sandhill Cranes wintering in this region are exposed to potentially stressful environmental conditions.

I measured the physiological state of different groups of cranes wintering in this arid region and exposed to different environmental conditions. I used fecal glucocorticoid metabolite analyses to measure stress hormone secretion (i.e., corticosterone metabolite levels) as an index of physiological stress (Wingfield et al. 1994b, Wasser et al. 2000; hereafter referred to, for brevity, as 'stress levels'). I expected cranes that winter in permanent large wetlands surrounded by abundant agricultural fields to experience lower stress levels than cranes wintering in temporary small wetlands surrounded by desert scrub. I also expected cranes that winter in extremely remote

wetlands to experience lower stress levels than cranes wintering in wetlands surrounded by human settlements and exposed to human presence. Finally, I expected stress levels to be higher at the end of winter due to resource shortages and behavioral changes in preparation for migration.

The goal of my study was to quantify stress levels in Sandhill Cranes wintering in northern Mexico. The objectives were to; (1) measure whether stress levels of Sandhill Cranes vary due to exposure to environmental stress factors, (2) measure whether stress levels of Sandhill Cranes vary due to exposure to human disturbance, and (3) determine if stress levels in Sandhill Cranes vary during winter.

## MATERIALS AND METHODS

### *Study Area*

I sampled a stress hormone of Sandhill Cranes in wetlands distributed within the wintering range in northern Mexico in the Chihuahuan Desert Ecoregion (Dinerstein et al. 2001; Fig. 1). The region covers 629,000 $km^2$ and stretches north-south from south-central United States to central Mexico, where it includes a portion of the state of Chihuahua, most of Coahuila, eastern Durango, northern Zacatecas, northern and central San Luis Potosí, and some small portion of Nuevo Leon and Tamaulipas (Fig. 1). The Chihuahuan Desert is bordered by the Sierra Madre Occidental to the west and the Sierra Madre Oriental to the east.

I collected my samples from wetlands historically used by cranes. Sandhill Cranes had been recorded in these wetlands by the U.S. Fish and Wildlife Service surveys since 1953 (Drewien et al. 1996). I selected six sites to represent a gradient of

resource availability for wintering cranes in the Chihuahuan Desert Ecoregion: (1) Laguna de Babícora (state of Chihuahua; Fig. 2), (2) Laguna de Mexicanos (state of Chihuahua; Fig. 3), (3) Laguna de Ojo Federico (state of Chihuahua; Fig. 4), (4) Laguna de Santiaguillo (state of Durango; Fig. 5), (5) Presa San Carlos de Mapimí (state of Durango; Fig. 6), and (6) Laguna de San Juan de Ahorcados (state of Zacatecas; Fig. 7; Table 1).

*Data Collection*

Sample collection/preservation.—I collected feces of Sandhill Cranes from roost sites around the six wetlands described above during the winter (October to February) of 2007/08 and 2008/09. I located roost sites by direct observation of flocks of cranes leaving at dawn. The condition of fecal samples that lay on the ground for a long period of time and are exposed to high temperatures can be compromised due to biochemical changes in immunoreactivity and degradation of steroids (Matkovics 1972, Terio et al. 2002). Therefore, I collected fresh samples that had been deposited during the morning hours to avoid microbes in the feces to start metabolizing the fecal glucocorticoids (Woods 1975, Mostl et al. 1999, Washburn and Millspaugh 2002). In addition, previous studies have detected that corticosterone levels vary with daily activity rhythms and with the time between excretions (Touma et al. 2003). The metabolic rate of birds drops more than normal during the night (i.e., when birds cannot forage) contributing to energy saving needed for the next morning (Astheimer et al. 1992). For that reason, the morning hours coincide with the maximal 24 h basal corticosterone concentrations (Carsia and Harvey 2000). I collected all samples early in the morning to avoid interference with diurnal activity rhythms that could mask stress measure. Another issue concerning

sample condition is the exposure to precipitation due to added moisture providing a suitable growth environment for microbes and detritivores (Washburn and Millspaugh 2002). Although rain is rare during the winter season in this area of Mexico, cranes roost in wetlands and deposit most of their feces in water. To ensure good quality samples, I only collected fresh feces (i.e., less than two hours after the cranes left their roost) that were deposited on the shores of wetlands.

I collected the samples using a 89 $cm^3$ sterile stainless steel scoop (AMS, American Falls, Idaho) and placed the samples in individual and sterile 118 $cm^3$ plastic whirl-pak bags (Nasco Whirl-Pak, Fort Atkinson, Wisconsin); numbered, dated, and assigned a location name for each one. I dipped and shook the scoop into a container filled with ethyl alcohol 90% after each collection to clean the scoop and avoid contamination between samples. I refilled the container with new alcohol between sites. Samples need to be frozen as quick as possible to preserve their fecal glucocorticoid metabolite (Terio et al. 2002, Millspaugh and Washburn 2004); therefore I used a 12 V AC/DC portable freezer (Engel Freezers, Jupiter, Florida) to keep the samples frozen while transportation between field and laboratory. I followed protocols of Millspaugh and Washburn (2004), and I froze my samples without adding any chemical treatment (e.g., acetic acid or ethanol: Khan et al. 2002, Lynch et al. 2003). I was then able to extract the fecal glucocorticoid metabolite at a later date, and the metabolite remained stable until I performed the analyses (Lynch et al. 2003). I stored the samples at –20 °C in the Laboratorio de Transgenesis Animal y Fertilización in Vitro of the Universidad Autónoma de Chihuahua for better preservation and to avoid fungal development until processing (Khan et al. 2002).

Remote landscape analyses.—I used spectral and spatial analyses of satellite images to estimate the size of the wetlands to indicate the amount of water available to cranes, to estimate the area of cropland to indicate the amount of food resources available to cranes, and to estimate the size of urban development to indicate human disturbance around each wetland (Jensen 2005). I downloaded current images from the Earth Science Data Interface (ESDI 2012) version 2.1.17 web application (Global Land Cover Facility, University of Maryland, College Park, Maryland) (Table 2). I used ArcGIS version 9.3 software (Environmental Systems Research Institute, Redlands, California) to process the images. I used Composite Bands from the Data Management ArcToolbox in ArcMap (ArcGIS) to create a single raster dataset from multiple bands. I used combinations of spectra typically used for these types of delineations. The best spectral band combination for the delineation of the wetland was 3, 2, and 1 (red: 0.63-0.69 µm, green: 0.52-0.60 µm, and blue: 0.45-0.51 µm, wavelengths respectively; Sheffield 1985). This is a natural color band combination with ground features appearing in colors similar to their appearance to the human eye. The best spectral band combination to represent agricultural fields was 4, 3, and 2 (near infrared: 0.75-0.90 µm, red: 0.63-0.69 µm, and green: 0.52-0.60 µm, wavelengths respectively; Sheffield 1985). This is a standard false color composite with ground features appearing in colors similar to traditional infrared aerial photography. With this combination of spectra, vegetation and farmland appears in shades of red; such an approach is used in studies of crop growth because areas in red are easily distinguished as productive agricultural fields (Jensen 2005). I projected the images using the World Geodetic System (WGS) 1984 datum and Universal Transverse Mercator (UTM) geographic coordinate system.

I created a GIS layer of polygons to determine the area occupied by each wetland in the satellite images. Wetlands in the Chihuahuan Desert Ecoregion tend to have variable water levels; wetlands fill to maximum capacity during the rainy season of July, August, and September. Wetlands are prone to evaporation, and thus reduce in size, during the dry season between October and June. In addition, some wetlands used by cranes are temporary and can dry completely by the end of winter. During dry periods when wetland water evaporates, crystallized salts form on the shores leaving white broad lines that can be seen from the satellite images. I used a series of historical images to find the extension of the minimum and maximum wetland sizes during recent time periods (i.e., previous 5-8 years) for each wetland. I created a 20-km buffer zone around the maximum wetland size polygon to delimit the foraging area potentially used by cranes (Iverson et al. 1985). Food abundance within a 20-km radius of the roost is one of the main environmental variables that influences crane distribution in winter grounds (Iverson et al. 1985); however, the maximum distance that cranes have been recorded to disperse from their roosting sites in search for food is 13 km (Iverson et al. 1985).

I then created a GIS layer of polygons to delineate the area of cropland inside the foraging area. Since the foraging area varied depending on wetland size, I obtained the proportion of cropland available to cranes by dividing the cropland area by the foraging area so that I could compare the abundance of food between wetlands (Fig. 8-13). The proportion of cropland became the food abundance variable in my models set. To verify that the types of crops identified from the satellite images corresponded to crop types that cranes were able to consume, I obtained official records from a government data base, the Sistema Estatal y Municipal de Bases de Datos, on the sown area of major crops (per

Mexican government: corn, oats, sorghum, alfalfa, wheat, beans, green chili, red tomatoes, and pastures) in each municipality (SIMBAD 2012; Fig. 14).

I used, as a size covariate in my models, the mean of the minimum and the maximum wetland area for each wetland (Fig. 8-13). I also used the distance between the centroid (i.e., the geometric center) of the maximum wetland size and the closest city of 10,000 habitants or more (measured by GIS) as a proxy for the level of human disturbance (Fig. 15). I settled on 10,000 people as the cut-off for my definition of a human settlement based on my assessment of sizes of cities near where cranes had been recorded in the past; very few cities in close proximity to cranes had populations greater than 10,000.

Predator surveys.—I used field surveys to estimate abundance of predators, including birds of prey and carnivores; I assumed that predator abundance correlated with disturbances of cranes by predators (Sutherland and Green 2004). Few birds of prey are large enough to predate on adult Sandhill Cranes; however, there are several accounts of smaller birds of prey such as Red-tailed Hawk (Dwyer and Tanner 1992, Heatley 2002, Olsen 2004) and Red-shouldered Hawk (*B. lineatus*) (Heatley 2002) attacking juvenile Sandhill Cranes, and Peregrine Falcon (*Falco peregrinus*) attacking juvenile Demoiselle Cranes (*Anthropoides virgo*; Johnsgard 1983). Adult cranes react to a hawk flying overhead with alarm calls and alert postures that become contagious to the cranes in the vicinity as a way to indicate danger (Layne 1981, Heatley 2002). Therefore, the presence of hawk-size birds of prey stimulates an antipredator response (Heatley 2002) sufficient to cause disturbance (pers.observ.). I estimated the density of birds of prey with surveys along line transects near my six wetland study sites during the winter (October to

February) of 2007/08 and 2008/09 (Burnham et al. 1980, Gregory et al. 2004, Braun 2005). I drove a vehicle along roads parallel to each wetland, and I traveled at a constant speed. Each transect covered the length of the wetland or 8 km if the wetland was < 8 km in length. I used two observers to cover both sides of the line and recorded every species of raptor using an identification field guide (Howell and Webb 1995). I recorded the perpendicular distance from each observation to the transect, the point along the transect, and the type of perch used by the bird. I conducted the transects once per month in the morning between 0600 and 0800 h.

I estimated the relative abundance of carnivores using scent station transects along the six wetlands described above during the winter (October to February) of 2008/09 (Linhart and Knowlton 1975, Conner et al. 1983, Diefenback et al. 1994, Wilson and Delahay 2001). I placed scent stations along the length of the wetland using a canine lure (Carman's Pro's Choice No. 3, New Milford, Pennsylvania) designed to attract Coyotes (*Canis latrans*), Fox species, and Bobcats (*Lynx rufus*). The lure also attracted feral carnivores that have the potential to disturb cranes, such as Domestic Dogs (*Canis lupus familiaris*) and Raccoons (*Procyon lotor*). Although raccoons have been reported to predate on captive cranes (Hartman 1987) there are no accounts of killings in the wild, however, there are accounts of disturbance of roosts at night (pers. observ.). I placed 4-6 drops of the lure on top of a rock and graded and brushed a 1 m diameter circumference of soil around it. In each transect, I positioned stations along the length of the wetland (5 to 8) spaced at 1-km intervals to ensure that the same individuals were not attracted to more than one station (Conner et al. 1983). I activated the scent stations in the afternoon and checked them the following morning for two nights monthly. I recorded a station as

visited when at least one track of a carnivore was identifiable in the circumference of fine soil around the rock.

Crane surveys.—I estimated Sandhill Crane abundance as a measure to quantify the potential for disturbance from other cranes (P. Pietz and D. Brandt, in litt.). I performed Sandhill Crane counts in the six wetlands described above during the winter (October to February) of 2007/08 and 2008/09 (Gregory et al. 2004). I conducted the counts twice monthly at sunset when cranes arrived to the roost and at sunrise when cranes left the roost with the help of a second observer. This double count provided a better estimate of the number of cranes using a wetland because some cranes arrive to the roost after sunset and some leave before sunrise. I then calculated the mean of all counts (Gregory et al. 2004) and used this as an index to crane abundance at each wetland.

*Data Analysis*

Hormone analyses.—In the laboratory, I thawed the fecal samples and processed them in a Petri dish with the use of a stainless steel surgical blade No. 11 (Feather Safety Razor, Osaka, Japan) and a pair of thin tweezers to separate small stones, sand, twigs, vegetation and soil particles from the samples. Avian fecal samples contain a white body of uric acid adhered to the excreta, which I assumed to be constant in proportion to the excreta volume across samples. Hormone metabolites are excreted in different amounts in urine and feces (Wasser et al. 2000). I assessed the excreta as a whole, following the protocols of Ludders et al. (2001) and Washburn et al. (2003) who suggested the technique provides a more complete estimate of total glucocorticoid metabolites (see Millspaugh and Washburn 2004, for critique).

The first step in the quantification of steroid hormones is the extraction of the hormone (e.g., corticosterone) from the biological medium where it is confined (e.g., plasma, urine, or excreta). I extracted the stress hormone from each fecal sample by placing the entire sample into a sterile 50 ml screw cap polypropylene conical bottom centrifuge tube (Corning, Tewksbury, Massachusetts) that had been previously weighed on an analytical precision balance (Explorer Ohaus, Parsippany, New Jersey) and numbered. I then weighed again the tube with the sample inside to determine the amount of hormone extraction buffer to be added. The extraction buffer consisted of 45% PBS (Dulbecco's Phosphate Buffered Saline powder, Grand Island, New York), 50% methanol (i.e., methyl alcohol), and 5% Tween-20 detergent solution (i.e., polyoxyethylene 20 sorbitan monolaurate) (Bauman and Hardin 1998). I added extraction buffer to each sample following a relation of 1 ml of buffer for every 0.2 g of sample. I placed the tube in a vortex mixer for 1 min until the sample had become well dispersed. I shook the sample for 4 h at 180 rpm until homogenized. Mixing is especially important because fecal glucocorticoid metabolites are not evenly distributed throughout the entire fecal mass (Wasser et al. 1996). Next I centrifuged the sample at 800 relative centrifugal force (RCF) and 0 °C for 10 min to separate the supernatant from the rest of the solid sample. I decanted the supernatant containing the steroid extract into a clean 15 ml screw cap conical bottom tube (Corning, Tewksbury, Massachusetts). I then froze the extract at -20 °C to stabilize samples until I could perform the assays.

To start the assays, I placed the tube with the remainder of the solid portion of the sample into an oven to dry overnight at 100 °C. Then, I allowed the tube to come to room temperature and weighed it with the dry matter on an analytical precision balance.

I then calculated the dry weight of the fecal sample by subtracting the weight of the empty tube recorded early on minus the weight of the dry tube with the solid residue (protocol modified from Shideler et al. 1993; J. Bauman, pers. comm.).

Once I extracted the hormone from the fecal sample, I performed an enzyme immunoassay (EIA) for each extraction. Most steroid analyses are performed using radioimmunoassays (RIAs) mainly because they are more precise than EIAs and because of tradition (Mostl et al. 2005). However, fecal glucocorticoid metabolite analyses are increasingly being done with EIA because of the cost of obtaining a custom made radioactive label and the import restrictions between countries. I therefore used a non-species specific commercially available corticosterone Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (Corticosterone Kit, Neogen Corporation, Lexington, Kentucky). This kit is used for quantitative analyses of corticosterone levels in biological fluids (for more details about this method and its validation see Chapter 3). ELISA's operate on the basis of competition between the label or enzyme conjugate (e.g., corticosterone horseradish peroxidase concentrate) and the steroid being measured (e.g., corticosterone) for a limited number of antibody binding sites on a coated plate (Kellie 1975). The label of the kit has a cross reactivity of 100% with the excreted corticosterone metabolites.

I added 40 samples and eight standards of known corticosterone concentration in duplicates to a 96-well corticosterone antibody coated microplate. I added enzyme conjugate to each well using an 8-multichannel pipette with a volume range from 30 to 300 µl (Eppendorf, Hamburg, Germany) to ensure the same time of reaction between wells and mixed it shaking gently with the use of a microplate shaker. I then let the microplate incubate at room temperature for one hour. The competition for the binding

sites takes place during the incubation period. After incubation I washed the microplate three times with a wash buffer to remove all the unbound material. I added a substrate (i.e., tetramethylbenzidine and hydrogen peroxide) with a multichannel pipette to react and develop color at room temperature for 30 min to detect the bound material left in the wells, and then I added 1N HCl to stop the enzyme reaction. I shook the microplate again to ensure uniform color throughout each well and read the plate using a microplate reader with a 650-nm filter. I used a microplate reader to measure the amount of light that is absorbed by the samples (i.e., optical density) and compare it with the amount absorbed by the standards.

For the calculations, I averaged the optical density readings of the duplicates of the standards and calculated the percent of maximal binding ($\%B/B_0$) by dividing the averages of each standard ($B_1$ to $B_7$) by the average of the standard with zero concentration of corticosterone ($B_0$) multiplied by 100. I calculated a standard curve between the percent of maximal binding and the concentration of corticosterone (ng/ml) of each standard and obtained a linear regression equation to calculate the concentration of corticosterone for each sample (Appendix B). When the concentration of a sample fell outside the standard curve I diluted the sample with a dilution factor (typically four) and repeated the assay, as per manufacturer instructions. I then multiplied the new concentration obtained for that sample by the dilution factor to calculate the final value.

Statistical analyses.—I used Distance 6.0 release 2 software (Thomas et al. 2010) to estimate the density ($\hat{D}$) of birds of prey at each wetland. My data from the transects consisted of perpendicular distance observations of single subjects recorded as from a single observer (Buckland et al. 2004). I combined repeated surveys over both seasons

and quantified bird abundance by estimating the density of birds (individuals/ha). I compared four standard models of detection probability: uniform, half-normal, hazard-rate, and negative exponential. And, I allowed program Distance to select the most appropriate adjustment to the key functions: cosine, and simple or Hermite polynomial. I then selected the best model using Akaike's Information Criterion (AIC) scores for each model (Zuur et al. 2007). When the AIC difference between models was smaller than two, I considered the models to be equivalent and selected the more simple one (Burnham and Anderson 2010). I eliminated models from consideration if they did not converge (Appendix A).

I used an index to quantify the abundance of carnivores from the data collected along each survey line. I recorded which scent stations had been visited every night and determined, for each site, the total number of visits. I calculated the total operative station-nights as a measure of catch by trapping effort, by multiplying the number of stations set in each transect by the number of nights the stations were active. Therefore, I calculated the index of relative abundance (IRA) of carnivores (Linhart and Knowlton 1975) in each wetland as follows:

$$\text{IRA} = \frac{\text{Total animal visits}}{\text{Total operative station–nights}} \times 1{,}000$$

I examined the levels of corticosterone response from Sandhill Cranes wintering in different locations using Generalized Linear Mixed Modeling (GLMM) in R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria; R Development Core Team 2011) and package lme4. I used the location (i.e., wetland) as a random effect, and

I created 36 competing, biologically meaningful, single- and multiple-factor models to explain variability of corticosterone levels: food abundance, wetland size, distance to city, predator density, number of cranes, month, and year. An initial graphical data exploration revealed that the response variable was not normally distributed. Therefore, I executed the analysis on log transformed corticosterone levels to retain normality of the response variable. I also standardized some of the explanatory variables using a *z* transformation to adjust them to a similar scale. I selected the best model using Akaike's Information Criterion (AIC) scores for each model (Zuur et al. 2007). When $\Delta AIC \leq 2$, I considered the models to be equivalent and selected the most parsimonious model (Burnham and Anderson 2010). When the best model had a large number of parameters, I used a Bayesian Information Criterion (BIC) to verify my selection. BIC introduces a higher penalty term for the number of parameters in the model (Zuur et al. 2007). I also estimated the relative importance of the variables in the selected models by summing the weights of the models where each variable occurred ($w_+(j)$). A larger sum of the weights indicated a more important variable, relative to the rest of the variables (Burnham and Anderson 2010).

**RESULTS**

Lagunas de Mexicanos and Santiaguillo had the highest food abundance with a proportion of cropland >0.300 inside the crane's foraging area. In contrast, Laguna de San Juan de Ahorcados and Presa San Carlos de Mapimí had the lowest food abundance with a proportion of cropland <0.100. Although Laguna de Mexicanos had less cropland area than Laguna de Babícora (664.5 vs. 800.6 $km^2$), the first had a higher proportion of cropland inside the crane's foraging area (0.356; Table 3). According to data obtained

from SIMBAD (2012), Gómez Farías, the municipality where Laguna de Babícora is located, had the highest percentage of landscape planted to corn (74%) and Nuevo Ideal, the municipality where Laguna de Santiaguillo is located, was the area with the highest percentage of oats (42%). The municipalities that host the wetlands with less proportion of cropland, Tlahualilo – home to Presa San Carlos de Mapimí, and General Francisco R. Murguía – home to Laguna de San Juan de Ahorcados, planted beans (57%) and alfalfa (21%) as major crops (Fig. 14). The majority of crops were useful to cranes except for alfalfa, beans, green chili, and red tomatoes (Chapter 4).

Wetland size ranged from 0.6 $km^2$ to 172.5 $km^2$, for Laguna de Santiaguillo and Presa San Carlos de Mapimí, respectively (Table 4). Presa San Carlos de Mapimí was not only the smallest wetland but also the most remote one where the closest significant human settlement was located >100 km from the wetland, followed by Laguna de San Juan de Ahorcados. In contrast, Laguna de Ojo Federico had the most potential for human disturbance with a major city <10 km away (Table 5).

I recorded a total of 416 birds of prey ($\hat{D}$ = 0.045 individuals/ha ± 0.008) during the winters of 2007/08 ($n$ = 186, $\hat{D}$ = 0.018 individuals/ha ± 0.006) and 2008/09 ($n$ = 230, $\hat{D}$ = 0.043 individuals/ha ± 0.013) in a total of 26 line transects. Laguna de Mexicanos had the highest number of birds of prey observations ($n$ = 195), but Laguna de Babícora had the largest density of birds of prey ($\hat{D}$ = 0.412 individuals/ha). Presa San Carlos de Mapimí had the lowest density ($\hat{D}$ = 0.006 individuals/ha). Of the six wetlands, the transects performed at Laguna de Ojo Federico had the highest detection probability (P = 97.0; Table 6). The most abundant bird of prey was the American Kestrel (*F. sparverius*,

$\hat{D}$ = 0.046 individuals/ha, *n* = 186) followed by the Red-tailed Hawk (*B. jamaicensis*, $\hat{D}$ = 0.009 individuals/ha, *n* = 110), Northern Harrier (*Circus cyaneus*, $\hat{D}$ = 0.008 individuals/ha, *n* = 83), White-tailed Kite (*Elanus leucurus*, $\hat{D}$ = 0.003 individuals/ha, *n* = 9), Cooper's Hawk (*Accipiter cooperii*, $\hat{D}$ = 0.002 individuals/ha, *n* = 4), and Harris's Hawk (*Parabuteo unicinctus*, $\hat{D}$ = 0.001 individuals/ha, *n* = 11). Other species with $\hat{D}$ < 0.001 individuals/ha included Osprey (*Pandion haliaetus*), Crested Caracara (*Caracara cheriway*), Merlin (*F. columbarius*), Prairie Falcon (*F. mexicanus*), Peregrine Falcon (*F. peregrinus*), Sharp-shinned Hawk (*Accipiter striatus*), White-tailed Hawk (*B. albicaudatus*), Ferruginous Hawk (*B. regalis*), and Bald Eagle (*Haliaeetus leucocephalus*). Although Golden Eagle (*Aquila chrysaetos*) was never recorded during the surveys, they were observed at Laguna de Mexicanos and Presa San Carlos de Mapimí. Great Horned Owl (*Bubo virginianus*) was recorded at night at Presa San Carlos de Mapimí and Laguna de San Juan de Ahorcados.

I operated 209 scent stations for carnivores during the survey, and recorded 80 animal visits. The mean index of relative abundance (IRA) for all the sites was 382 ± 27 (*n* = 6; range: 321-500). Combining all the surveys for each wetland, Laguna de San Juan de Ahorcados had the highest relative abundance (IRA = 500) and Laguna de Santiaguillo had the lowest (IRA = 321; Fig. 16). The surveys only recorded Coyotes, Bobcats, and Domestic Dogs which were identified through track observations. In addition, Coyotes were directly observed twice while setting the transects at Laguna de Ojo Federico and once at Presa San Carlos de Mapimí.

I counted 59,875 Sandhill Cranes during the winters of 2007/08 ($n$ = 27,487) and 2008/09 ($n$ = 32,388). The mean number of individuals counted at each wetland was 1,460±390, and ranged from 0 to 12,614 cranes. Wetlands located in the state of Chihuahua had the highest number of cranes, including Laguna de Babícora (mean = 3,839±2,375), Laguna de Mexicanos (mean = 2,843±908), and Laguna de Ojo Federico (mean = 1,501±146). Wetlands located south in the states of Durango and Zacatecas had fewer cranes, including Presa San Carlos de Mapimí (mean = 602±207), San Juan de Ahorcados (mean = 210±55), and Laguna de Santiaguillo (mean = 185±105).

I collected 331 fresh fecal samples in all study sites during the winters of 2007/08 ($n$ = 135) and 2008/09 ($n$ = 196). In the lab, I run a total 13 ELISA's for 520 fecal samples after repeating samples that needed dilution. The mean fecal glucocorticoid concentration during the study period was 301.3±50.9 ng/g dry feces ($n$ = 320, median = 111.9). A visual inspection of the corticosterone results suggested some values were outliers; therefore, I removed improper samples beyond three standard deviations of the mean accounting for 3% of the total samples (Li and Wong 2001). Corticosterone levels varied among the six wetlands included in the study ($F_{5, 314} = 8.2$, $P < 0.0001$) when pooling data from both years; two of the wetlands (Presa San Carlos de Mapimí and Laguna de Ojo Federico) recorded higher concentrations of corticosterone than the rest of the wetlands (Fig. 17). Corticosterone levels were higher throughout January and February than November and December ($F_{3, 316} = 5.4$, $P = 0.001$) when pooling data across locations in both years (Fig. 18). Stress levels decreased with wetland size ($\beta_{WetS}$ = -0.409, SE = 0.085, 95% CI: -0.579 to - 0.238) and time of sampling (Fig. 19 and 20). Although the selected best model had a weight of approximately one, indicating a great

model selection certainty, it also had a considerable amount of parameters ($k$ = 15; Table 7). In support, the BIC (85.7) criterion also selected that best model to describe the data regardless of the number of parameters. The second best model indicated that human disturbance was not an important factor to explain changes in corticosterone levels ($\beta_{CityD}$ = -0.008, SE = 0.123, 95% CI: -0.255-0.239). The time of sampling (i.e., month) was the only single factor present in the top ten models ($w_+$(Month) ≈ 1). The season in which the samples were collected (i.e., year) was also important ($w_+$(Year) ≈ 1), followed by the size of the wetland ($w_+$(WetS) ≈ 1). The most important variable not included in the best model was distance to a large city; however, the weight was substantially lower relative to the previous variables ($w_+$(CityD) < 0.0001).

## DISCUSSION

This is the first study to measure the effects of environmental conditions on the physiological state of cranes in the wild. I showed that the physiological stress level of Sandhill Cranes in Mexico is mainly affected by the time of winter and the size of wetlands at their wintering sites.

Wetland size.—The results of my study confirmed that cranes wintering in small wetlands had higher concentrations of corticosterone, and thus were under more stress than cranes wintering in large wetlands with abundant water resources. The effects of water restriction have a combined impact on the physiology and behavior of the animals that suffer the consequences. Such impacts have been well studied in laboratory controlled conditions involving several taxa. Li et al. (2000) reported changes in plasma cortisol concentrations in domestic sheep (*Ovis aries*), affecting metabolic rate and

energy conservation. Dunlap (1995) reported corticosterone changes in Western Fence Lizards (*Sceloporus occidentalis*) and changes in activity level as well. Similar effects were recorded with passerine species exposed to water deprivation such as White-crowned (*Zonotrichia leucophrys*; Lynn et al. 2003) and Song Sparrows (*Z. melodia*), Pine Siskins (*Carduelis pinus*), and Dark-eyed Juncos (*Junco hyemalis*); they all had higher corticosterone and activity levels (Astheimer et al. 1992). However, few studies have investigated the effects of aridity on stress hormones in the wild but all of them have found that water shortage during dry seasons produced higher levels of glucocorticoids. Many of these studies have examined mammals such as Baboons; Yellow (*Papio cynocephalus*; Gesquiere et al. 2008), Olive (*P. anubis*; Sapolsky 1986), and Chacma (*P. ursinus*; Weingrill et al. 2004) Baboons; Ring-tailed Lemurs (*Lemur catta*; Cavigelli 1999); and a few bird species such as Lapland Longspurs (*Calcarius lapponicus*), Redpolls (*Carduelis flamea*), and Snow Buntings (*Plectrophenax nivalis*) (Romero et al. 2000).

Wetlands in my study area varied greatly in terms of size and seasonality. Cranes in Mexico spend about five months of the year in a very arid region known as the Chihuahuan Desert with a mean annual rainfall between 125 and 400 mm (Ferrusquia-Villafranca et al. 2005). Despite its arid condition, the Chihuahuan Desert has multiple wetlands scattered through its extension that contain a high degree of freshwater endemic biota (Dinerstein et al. 2001) and has been identified as one of the most important ecoregions in the world (Olson and Dinerstein 1998). However, wetlands have been heavily decimated due to excessive water diversion for agriculture and degraded soil and many perennial streams and springs are now only seasonally wet (Dinerstein et al. 2001).

My data suggests that these changes to the landscape can have measurable effects on cranes. Wetlands fill to maximum capacity during the rainy season of July, August, and September, and are prone to evaporation, and thus reduce in size, during the dry season between October and June. In Mexico, wetlands represent critical habitat for cranes; during the night they use them as roost sites and during the hottest hours of the day they use them as rest sites and source of drinking water. Without wetlands of a substantial size, cranes would probably stop using this region as their wintering grounds.

Sandhill Cranes in Mexico are not only affected by water shortage but also by water unpredictability, a characteristic of arid environments (Bauder 2005). Some wetlands used by cranes are temporary and can dry completely by the end of winter. Small wetlands, by definition, are most prone to drying events. Resource unpredictability (e.g., water) can also affect corticosterone levels (Lucas et al. 2006). In fact, Romero (2004) defined an environmental stressor as an unpredictable stimulus that causes a stress response in an animal. My data supported this argument: cranes wintering in temporary wetlands, that sometimes become dry by the end of the winter, exhibited higher levels of stress hormones than cranes wintering in large enough water bodies that cannot dry over winter.

Therefore, water restrictions have an effect on behavior and physiology that is mediated by an increase in corticosterone. Such increase triggers a rise of the metabolic rate and activity level (Astheimer et al. 1992) to aid in the search of resources through mobilization of energy to be able to maintain the allostatic load. The allostatic load is known as the daily and seasonal energy requirements of an organism to obtain food or water resources plus the extra energy needed for other activities including to find a mate,

breed, migrate, or avoid predators (McEwen and Wingfield 2003). A reduction in the allostatic load results in a decrease of fecal glucocorticoids because there is no need for behavioral and physiological processes to be activated when the risk for chronic stress is not present (Wingfield et al. 1998). I predict cranes that winter in small wetlands to fly longer to forage (Chapter 4) than cranes that winter in large wetlands due to an increase in corticosterone levels.

Some studies have also explored the effects of a combination of water deprivation and heat stress in both domestic and wild animals. Heat stress in lambs caused increased respiration rate, body temperature, and plasma cortisol levels; however, dehydration had a bigger effect on cortisol increase than heat (Lowe et al. 2002). Baboons in an African semiarid environment also exhibited higher levels of fecal glucocorticoid during the dry season and during months of high average maximum temperatures (Gesquiere et al. 2011). Although the time of the year that cranes are in Mexico is not the hottest season, temperatures can still reach levels close to the avian upper critical limit (i.e., around 40°C; Gill 1999) forcing them to return to water bodies to cool down as an adaptation to reduce body temperature (pers. observ.). I recorded these circumstances towards the end of February and in the middle of the day when temperatures reached a maximum of 38°C. The combination of hotter and drier conditions may explain the tendency of an increase in corticosterone levels towards the end of the winter during my study.

In fact, time of year of sample collection was the most important single factor to explain concentration of stress hormones in feces. Seasonal variation in corticosterone levels have been well documented in field studies and tend to be highest in winter (Lucas et al. 2006). Such seasonal changes in stress hormones follow physiological changes that

are independent of local conditions (Romero et al. 1997). Other studies have reported similar findings in Sonoran Desert breeding birds, where well adapted species reactivated their adrenocortical stress response after breeding to cope with winter desert conditions (Wingfield et al. 1992). Therefore, I expected corticosterone levels to be higher at the end of my study period, not only because of resource shortages but also because of observed behavioral changes such as migratory restlessness and pair bonding in preparation for the breeding season (pers. observ.). Sandhill Cranes become social animals during winter and spring migration (Tacha 1988). Although living in social groups has many advantages (e.g., cooperation and social support), it also has some disadvantages such as having to cope with a higher allostatic load due to social conflict and competition (Goymann and Wingfield 2004). Romero (2004) identified increased aggressive interactions in social animals as a cause of increased corticosterone levels. Although I did not collect data to test whether competition existed among cranes, social foraging theory predicts increased competition for limited food resources in wintering flocks (Giraldeau and Caraco 2000). Hence, the tendency of increase in corticosterone levels with time that I observed during my study is probably due to a combination of factors including hotter and drier environmental conditions, increased competition due to fewer food resources, and preparation for spring migration.

Disturbance.—Disturbance did not play a role in steroid concentration in cranes wintering in Mexico. Sources of disturbance could be classified as non-anthropogenic (i.e., abundance of birds of prey, carnivores, and cranes), and anthropogenic (i.e., proximity to large cities). Only human disturbance estimated as the proximity to a large city appeared in the second best model; however, its effect on stress hormones was not

even considered due to the small weight of the model. Previous studies have shown that human noise and disruption caused by heavy machinery has a direct effect on glucocorticoids in wild animals (Millspaugh et al. 2001, Creel et al. 2002, Pereira et al. 2006). Human disturbance is a type of stimuli perceived by wild animals as a form of predation risk (Frid and Dill 2002) and they react to human presence as they would in front of a potential predator (Beale and Monaghan 2004). Although in general cranes tend to search for remote and isolated wetlands to roost (Lovvorn and Kirkpatrick 1981), they occasionally become accustomed to human activities and roosts may exist along busy areas (Littlefield 1986). In areas of high quality habitat (e.g., where food is abundant and close to the wetland), roosting cranes will tolerate human disturbance including nearby powerlines, seldom-used roads, or railroads (Sparling and Krapu 1994). However, Sandhill Cranes in Mexico do not tolerate human presence (pers. observ.) and behave different from places such as Florida and Nebraska (Cooper 1996). The fact that human presence did not have an effect on stress levels in my study area suggests that Sandhill Cranes may avoid areas with constant human disturbance. In fact, roosting cranes have been known to avoid areas with intrusive human disturbance such as hunting (Lovvorn and Kirkpatrick 1981). Although hunting is only allowed in designated areas in Mexico and few people have access to hunting equipment, it was observed in Laguna de Ojo Federico and in Laguna de Babícora; in both locations, cranes abandoned the area and temporarily moved to an adjacent valley.

Regarding natural sources of disturbance such as predator density, previous studies have reported a spike in corticosterone levels when animals are exposed to the presence of a predator (Thaker et al. 2009). Such increase is supposed to facilitate a

behavioral reaction needed for survival (Sapolsky et al. 2000). However, density of birds of prey did not have an effect on corticosterone levels of Sandhill Cranes. Wetlands with bigger cropland area had a higher raptor density estimate. This was probably due to a higher population density of rodents usually associated with agricultural areas (Stenseth et al. 2003). The use of line transects to estimate abundance of raptors was adequate to assess disturbance from large enough species (i.e., mainly hawks) to provoke flocks of cranes to flight (pers. observ.); however, it was not the best method to estimate abundance of crane predators. Only Golden Eagles (Walkinshaw 1949, Johns 1977, Littlefield 1986, Bizeau et al. 1987, Cannings et al. 1987, Littlefield and Lindstedt 1992, Ellis et al. 1999a), Great Horned Owls (Hartman 1987; D.A. Brandt, pers. comm.), and possibly Bald Eagles (Walkinshaw 1949, Cooper 1996) have been reported to attack adult size Sandhill Cranes. Golden Eagles are most likely the main bird capable of predating on cranes and have also been reported to attack other species such as Whooping (Windingstad et al. 1981), Demoiselle (Thiollay 1979, Ellis et al. 1999b), and Common Cranes (*G. grus*) (Tjernberg 1981, Muñoz-Pulido et al. 1993, Watson 1997). A closely related species, the Imperial Eagle (*A. heliaca*) has also been reported to predate on several species of cranes (Katzner et al. 2006). Golden Eagles were always observed outside the line transects indicating that a different survey method should have been used to record and estimate their abundance.

In this part of Mexico there are five sympatric species of wild carnivores, including Coyotes, Kit (*Vulpes macrotis*) and Gray (*Urocyon cinereoargenteus*) Foxes, Cougar (*Puma concolor*) and Bobcats (Ceballos et al. 2002), although the surveys did not record any fox or Cougar. I did not expect to record any species of foxes because the

habitat surrounding each study wetland was agriculture, dry scrub, and grassland. Gray Foxes occur in semi-arid areas of northern Mexico where forest or thick brush cover is sufficient (Harrison 1997) and Kit Foxes inhabit arid and semi-arid regions associated with Black-tailed Prairie Dog (*Cynomys ludovicianus*) town complexes (McGrew 1979) only remaining in isolated areas of northern Chihuahua (Ceballos et al. 1992). The main mammalian predator of cranes, the Coyote (Cooper 1996), was adequately estimated through scent station transect lines. Coyotes have been known to take adult cranes, especially in areas with dense vegetation where the carnivores can hide (Littlefield 1986). Although in a study of coyote diet in the Chihuahuan Desert (Hernandez et al. 2002), birds did not represent an important prey item, bird remains frequencies' increased during the winter when species abundance increase due to migratory arrivals. Even though predation by coyotes does not occur frequently (Nesbitt and Badger 1995), disturbance by both Coyotes and domestic dogs does occur often (pers. observ.). In my study I pretended to record density of carnivores as a measure of disturbance rather than 'predation pressure' on cranes. However, density of carnivores did not have a significant effect on stress levels either. Though I recorded a high presence of mammalian predators in each wetland, Laguna de San Juan de Ahorcados had a much higher density, and it is in fact, a wetland where I recorded high levels of corticosterone. The rest of the sites had fairly similar carnivore densities that did not incurred disturbance to roosting cranes. My results were higher than other studies performed in western United States (Linhart and Knowlton 1975), yet their maximum density in south Texas was the same that I obtained from Laguna de San Juan de Ahorcados (RIA = 500). My results were also higher than a study performed in southern Mexico (Monroy-Vilchis and Velázquez 2002) where their

average index reached 228 versus 382 in my study sites. I expected to find higher densities of carnivores in my study area because the remote desert shrubby landscape is prime habitat for Coyotes (Gese et al. 2008).

Cranes roosting together in large numbers at night can easily disturb each other and force a flight. Disturbance from cranes has not been well documented in the past but recent research has identified crane abundance as a main cause of nocturnal disturbance to cranes themselves in the Platte River in Nebraska (P. Pietz and D. Brandt, unpublished data). However, crane abundance (i.e., number of cranes in the wetland) did not have a significant effect on corticosterone levels in my study. The sites with higher concentration of birds occurred in larger wetlands with internal sand banks, suggesting that roost space may be a limiting factor for smaller wetlands. However, the distribution of cranes in northern Mexico is most likely mediated by food availability. Wetlands used by Sandhill Cranes along their distribution range had differences in terms of crop availability within the delineated buffer zone. Wetlands in the south had less cropland than wetlands in the north. The greater availability of food surrounding northern wetlands could explain why more cranes concentrate in the northern states of Chihuahua and Durango than in any other state in Mexico (Drewien et al. 1996, Perez-Arteaga et al. 2005, Lopez-Saut et al. 2011).

Food abundance.—Contrary to what I expected, food abundance did not affect corticosterone concentrations in fecal samples. Food abundance has been negatively correlated with glucocorticoid metabolite concentrations in both mammals and birds. Foley et al. (2001) reported that free-ranging African elephants had increased cortisol during the dry season as food and body condition declined. In birds, corticosterone levels

of Carolina (*Poecile carolinensis*; Lucas et al. 2006) and Mountain Chickadees (*P. gambeli*; Pravosudov et al. 2001), and Black-legged (*Rissa tridactyla*; Kitaysky et al. 1999) and Red-legged Kittiwakes (*R. brevirostris*; Kitaysky et al. 2001) increased with food deprivation. Other studies have also shown that when birds have access to predictable and abundant food resources, they reduce their allostatic load (Goymann and Wingfield 2004). I expected cranes wintering in wetlands with low food abundance to have lower body mass, although I did not collect data on body condition. In fact, a combination of low body mass and high corticosterone levels have been reported as indicators of low food availability in birds (Lucas et al. 2006). In my study area, cranes wintering in wetlands with low food resources responded by moving geographically to a location where food (i.e., corn) was available (Chapter 4). These birds probably expended more energy to obtain their food by flying longer distances than birds that had abundant corn fields nearby. In my study, I was able to distinguish between wetlands with different food abundance but I was not able to detect changes in food abundance throughout the season. It is possible that by the end of winter, most waste grain in the fields has been consumed and food abundance decreases significantly. This could also explain the increase in stress hormones towards the end of winter.

Summary.—Although the use of fecal glucocorticoid metabolite measure to address stress responses in wild populations has great potential for the field of conservation biology, there is a considerable amount of confounding factors that complicate the interpretation and application of results (Millspaugh and Washburn 2004). The nature of these confounding factors can be divided into controllable factors such as sampling issues and assay artifacts, and uncontrollable or biological factors. Biological

factors such as sex, age, reproductive status, daily rhythms, seasonal patterns, diet, and excretion route, can influence the adrenocortical activity of a wild animal making the interpretation of fecal glucocorticoid metabolites even more challenging (Millspaugh and Washburn 2004). To address this potential issue, I collected enough samples to obtain a representation of the physiological status of the population of cranes wintering in the area in general. Another problem faced by this technique is the lack of basal fecal glucocorticoid metabolite values in wild conditions. Without these values, which could be interpreted as 'normal values', it is difficult to assess whether results are elevated or not making it difficult to relate levels of glucocorticoids to distress in wild populations (Millspaugh and Washburn 2004). In my study, since I did not have access to basal values for Sandhill Cranes in the wild, I limited my interpretations to comparisons between groups of cranes exposed to different environmental conditions.

My data suggests that cranes wintering in wetlands with scarce water resources maintained a higher concentration of fecal corticosterone throughout the winter. Higher stress levels could represent the mechanism that helps these birds survive the winter by increasing their metabolic rate and activity level in search of resources (Astheimer et al. 1992). Higher stress levels could also be an indication that human modifications to the landscape (i.e., wetland habitat) can cause a species to shift its behavior to adapt to new conditions. Conservation of wetlands in northern Mexico is crucial for the continuity of the species in this arid region. Sandhill Cranes depend heavily on wetland habitat and are susceptible to water changes. Habitat requirements of cranes can be met by preserving wetlands and an agricultural buffer zone around them. However, there is a direct conflict between cranes and farmers' interests both in terms of water usage and crop availability

(Chapter 5). Habitat availability has been identified as the most important limiting factor for Sandhill Crane populations (Cooper 1996); therefore, if one of the mandates of the Mexican government is to preserve its biodiversity (CONABIO 1998), it needs to prioritize wetland conservation as a primary management option. Some of the suggestions for wetland's conservation proposed in the past by several authors (Iverson et al. 1987, Dwyer and Tanner 1992) are also valid for this region, such as maintaining the structural integrity of wetlands, controlling water use permits, avoiding building dykes, and providing incentives to discourage draining for agricultural purposes are some of the examples.

Wintering habitat in Mexico may represent a maintenance-type environment that allows cranes to sustain their body weight until they reach the staging grounds. On the one hand, Sandhill Cranes are benefiting from an increase in agricultural practices and are being recorded in new sites (Lopez-Saut et al. 2011) but on the other hand, such practices are draining the water resources needed by the cranes. In addition, not only availability but also predictability of water resources in the future will play an important role in the status of the species in the region (Pravosudov et al. 2001). Historically, cranes used to winter into central Mexico (Leopold 1965) but changes in agricultural practices and habitat loss have restricted the species to winter only in northern Mexico. Therefore, we have already observed a shift in crane distribution when habitat becomes unsuitable suggesting that when conditions pass a certain threshold (e.g., wetland desiccation), cranes can no longer acclimate to the conditions by maintaining high stress levels and they eventually abandon such wintering sites (pers. observ.). Although Sandhill Cranes are extremely adaptable and the Mid-continent Population continues to

increase (Sharp and Vogel 1991), I predict that in the long term the species will decrease in northern Mexico as habitat conditions degrade due to a combination of water usage and climate change.

**LITERATURE CITED**

Andersen, D. E., O. J. Rongstad, and W. R. Mytton. 1989. Response of nesting red-tailed hawks to helicopter overflights. Condor 91:296-299.

Andersen, D. E., O. J. Rongstad, and W. R. Mytton. 1990. Homerange changes in raptors exposed to increased human activity levels in southeastern Colorado. Wildlife Society Bulletin 18:134-142.

Astheimer, L. B., W. A. Buttemer, and J. C. Wingfield. 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scandinavica 23:355-365.

Baltic, M., S. Jenni-Eiermann, R. Arlettaz, and R. Palme. 2005. A noninvasive technique to evaluate human-generated stress in the black grouse. Annals of the New York Academy of Sciences 1046:81-95.

Bauder, E. T. 2005. The effects of an unpredictable precipitation regime on vernal pool hydrology. Freshwater Biology 50:2129-2135.

Bauman, J. E., and A. Hardin. 1998. Measurement of steroids in animal feces with commercially available RIA kits intended for use in human serum. Journal of Clinical Lingand Assay 21:83.

Beale, C. M., and P. Monaghan. 2004. Behavioural responses to human disturbance: a matter of choice? Animal Behaviour 68:1065-1069.

Bizeau, E. G., T. V. Schumacher, R. C. Drewien, and W. M. Brown. 1987. An experimental release of captive-reared Greater Sandhill Cranes. Pages 78-88 *in* Proceedings 1985 Crane Workshop. Platte River Whooping Crane Habitat Maintenance Trust and U.S. Fish and Wildlife Service, Grand Island, Nebraska.

Braun, C. E. 2005. Techniques for wildlife investigations and management. The Wildlife Society, Bethesda, Maryland.

Buckland, S. T., D. R. Anderson, K. P. Burnham, J. L. Laake, D. L. Borchers, and L. Thomas. 2004. Advanced distance sampling. Oxford University Press, Oxford, UK.

Burnham, K. P., and D. R. Anderson. 2010. Model selection and multi-model inference: a practical information-theoretic approach. 2nd edition. Springer, New York, NY.

Burnham, K. P., D. R. Anderson, and J. L. Laake. 1980. Estimation of density from line transect sampling of biological populations. Wildlife Monographs 72:3-202.

Cannings, R. A., R. J. Cannings, and S. G. Cannings. 1987. Birds of the Okanagan valley, British Columbia. Royal British Columbia Museum, Victoria, BC.

Carrera, E., and G. de la Fuente. 2003. Inventario y clasificacion de humedales en Mexico. Parte I. Ducks Unlimited de Mexico, A.C., Mexico.

Carsia, R. V., and S. Harvey. 2000. Adrenals. Pages 489-537 *in* G. Causey Whittow, editor. Sturkie's Avian Physiology. Academic Press, San Diego, CA.

Cavigelli, S. A. 1999. Behavioural patterns associated with faecal cortisol levels in free-ranging female ring-tailed lemurs, *Lemur catta*. Animal Behaviour 57:935-944.

Ceballos, G., J. Arroyo-Cabrales, and R. A. Medellin. 2002. The mammals of Mexico: composition, distribution, and conservation status. Occasional Papers. Museum of Texas Tech University 218:1-27.

Ceballos, G., E. Mellink, and L. R. Hanebury. 1992. Distribution and conservation status of prairie dogs *Cynomys mexicanus* and *Cynomys ludovicianus* in Mexico. Biological Conservation 63:105-112.

Cockrem, J. F., and B. Silverin. 2002. Sight of a predator can stimulate a corticosterone response in the great tit (*Parus major*). General and Comparative Endocrinology 125:248-255.

CONABIO. 1998. La diversidad biologica de Mexico: Estudio de Pais, 1998. Comision Nacional para el Conocimiento y Uso de la Biodiversidad. Mexico.

Conner, M. C., R. F. Labisky, and J. D. R. Progulske. 1983. Scent-station indices as measures of population abundance for bobcats, raccoons, gray foxes, and opossums. Wildlife Society Bulletin 11:146-152.

Cooper, J. M. 1996. Status of the Sandhill Crane in British Columbia.*in* M. o. Environment, editor. BC Environment, Victoria, BC.

Creel, S., N. M. Creel, and S. Monfort. 1997. Radiocollaring and stress hormones in African wild dogs. Conservation Biology 11:544-548.

Creel, S., J. E. Fox, A. Hardy, J. Sands, B. Garrott, and R. O. Peterson. 2002. Snowmobile activity and glucocorticoid stress response in wolves and elk. Conservation Biology 16:809-814.

Delaney, D. K., T. G. Grubb, P. Beier, L. L. Pater, and M. H. Reiser. 1999. Effects of helicopter noise on Mexican spotted owls. Journal of Wildlife Management 63:60-76.

Delguidice, G. D., L. D. Mech, and U. S. Seal. 1989. Physiological assessment of deer populations by analysis of urine in snow. Journal of Wildlife Management 53:284-291.

Diefenback, D. R., M. J. Conroy, R. J. Warren, W. E. James, L. A. Baker, and T. Hon. 1994. A test of the scent-station survey technique for bobcats. Journal of Wildlife Management 58:10-17.

Dinerstein, E., D. Olson, J. Atchley, C. Loucks, S. Contreras-Balderas, R. Abell, E. Iñigo, E. Enkerlin, C. Williams, and G. Castilleja. 2001. Ecoregion-Based Conservation in the Chihuahuan Desert: A Biological Assessment. WWF, CONABIO, TNC, PRONATURA Noreste, and ITESM.

Drewien, R. C., W. M. Brown, and D. S. Benning. 1996. Distribution and abundace of sandhill cranes in Mexico. Journal of Wildlife Management 60:270-285.

Dunlap, K. D. 1995. Hormonal and behavioral responses to food and water deprivation in a lizard (Sceloporus occidentalis): implications for assessing stress in a natural population. Journal of Herpetology 29:345-351.

Dunlap, K. D., and J. J. Schall. 1995. Hormonal alterations and reproductive inhibition in male fence lizards (*Sceloporus occidentalis*) infected with the malarial parasite *Plasmodium mexicanum*. Physiological Zoology 68:608-621.

Dwyer, N. C., and G. W. Tanner. 1992. Nesting success in Florida sandhill cranes. The Wilson Bulletin 104:22-31.

Ellis, D. H., K. R. Clegg, J. C. Lewis, and E. Spaulding. 1999a. Golden eagle predation on experimental sandhill and whooping cranes. The Condor 101:664-666.

Ellis, D. H., P. Tsengeg, P. Whitlock, and M. H. Ellis. 1999b. Predators as prey at a golden eagle *Aquila chrysaetos* eyrie in Mongolia. Ibis 141:139-158.

ESDI. 2012. Earth Science Data Interface. Global Land Cover Facility. University of Maryland, College Park, Maryland. [http://glcfapp.glcf.umd.edu:8080/esdi/index.jsp].

Fernandez, C., and P. Azkona. 1993. Human disturbance affects parental care of marsh harriers and nutritional status of nestlings. Journal of Wildlife Management 57:602-608.

Ferrusquia-Villafranca, I., L. I. Gonzalez, and J. E. Cartron. 2005. Northern Mexico's landscape, part I: the physical setting and constraints on modeling biotic evolution Pages 11-28 *in* J. E. Cartron, G. Ceballos, and R. S. Felger, editors. Biodiversity, ecosystems, and conservation in northern Mexico. Oxford University Press, Inc., New York.

Foley, C. A. H., S. Papageorge, and S. K. Wasser. 2001. Noninvasive stress and reproductive measures of social and ecological pressures in free-ranging African elephants. Conservation Biology 15:1134-1142.

Frid, A., and L. Dill. 2002. Human-caused disturbance stimuli as a form of predation risk. Conservation Ecology 6:11-27.

Gese, E. M., M. Bekoff, W. Andelt, L. Carbyn, and F. Knowlton. 2008. *Canis latrans*. IUCN Red List of Threatened Species 2012.1.

Gesquiere, L. R., M. Khan, L. Shek, T. L. Wango, E. O. Wango, S. C. Alberts, and J. Altmann. 2008. Coping with a challenging environment: effects of seasonal variability and reporductive status on glucocorticoid concentrations of female baboons (*Papio cynocephalus*). Hormones and Behavior 54:410-416.

Gesquiere, L. R., P. O. Onyango, S. C. Alberts, and J. Altmann. 2011. Endorcrinology of year-round reproduction in a highly seasonal habitat: environmental variability in testosterone and glucocorticoids in baboon males. American Journal of Physical Anthropology 144:169-176.

Gill, F. B. 1999. Ornithology. 2nd edition. W. H. Freeman and Company, New York City, New York, USA.

Giraldeau, L.-A., and T. Caraco. 2000. Social Foraging Theory. Princeton University Press, Princeton, New Jersey.

Goymann, W. 2005. Noninvasive monitoring of hormones in bird droppings: Physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. Annals of the New York Academy of Sciences 1046:35-53.

Goymann, W., E. Mostl, and E. Gwinner. 2002a. Corticosterone metabolites can be measured noninvasively in excreta of European stonechats (*Saxicola torquata rubicola*). Auk 119:1167-1173.

Goymann, W., E. Mostl, and E. Gwinner. 2002b. Non-invasive methods to measure androgen metabolites in excrements of European stonechats, *Saxicola torquata rubicola*. General and Comparative Endocrinology 129:80-87.

Goymann, W., and J. C. Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Animal Behaviour 67:591-602.

Gregory, R. D., D. W. Gibbons, and P. F. Donald. 2004. Bird census and survey techniques. Pages 17-55 *in* W. J. Sutherland, I. Newton, and R. E. Green, editors. Bird Ecology and Conservation: A Handbook of Techniques. Oxford University Press, Oxford, UK.

Grubb, T. G., and R. M. King. 1991. Assessing human disturbance of breeding bald eagles with classification tree models. Journal of Wildlife Management 55:500-511.

Hadley, M. E. 1996. Endocrinology. Prentice-Hall, Upper Saddle River, New Jersey, USA.

Harper, J. M., and S. N. Austad. 2000. Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. Physiological and Biochemical Zoology 73:12-22.

Harrison, R. L. 1997. A comparison of gray fox ecology between residential and undeveloped rural landscapes. Journal of Wildlife Management 61:112-122.

Hartman, L. M. 1987. Summary of mortality of 14 species of cranes at The International Crane Foundation, 1972-1982. Pages 555-570 *in* Proceedings of the 1983 International Crane Workshop. International Crane Foundation, Baraboo, Wisconsin.

Hartup, B. K., G. H. Olsen, and N. M. Czekala. 2005. Fecal corticoid monitoring in whooping cranes (*Grus americana*) undergoing reintroduction. Zoo Biology 24:15-28.

Hartup, B. K., G. H. Olsen, N. M. Czekala, J. Paul-Murphy, and J. A. Langenberg. 2004. Levels of fecal corticosterone in sandhill cranes during a human-led migration. Journal of Wildlife Diseases 40:267-272.

Harvey, S. J., and T. R. Hall. 1990. Hormones and stress in birds: activation of the hypothalamopituitary-adrenal axis. Pages 453-460 *in* Progress in comparative endocrinology: proceedings of the eleventh international symposium on comparative endocrinology. Willy-Liss, Inc., New York.

Harvey, S. J., J. G. Phillips, A. H. Rees, and T. R. Hall. 1984. Stress and adrenal function. Journal of Experimental Zoology 232:633-645.

Heatley, J. J. 2002. Antipredator conditioning in Mississippi Sandhill Cranes (*Grus canadensis pulla*). Texas A&M University.

Hernandez, L., R. R. Parmenter, J. W. Dewitt, D. C. Lightfoot, and J. W. Laundre. 2002. Coyote diets in the Chihuahuan Desert, more evidence for optimal foraging. Journal of Arid Environments 51:613-624.

Howell, S. N. G., and S. Webb. 1995. A guide to the birds of Mexico and northern central America. Oxford University Press, New York.

Iverson, G. C., P. A. Vohs, and T. C. Tacha. 1985. Habitat use by sandhill cranes wintering in western Texas. Journal of Wildlife Management 49:1074-1083.

Iverson, G. C., P. A. Vohs, and T. C. Tacha. 1987. Habitat use by mid-continent sandhill cranes during spring migration. Journal of Wildlife Management 51:448-458.

Jensen, J. R. 2005. Introductory digital image processing: a remote sensing perspective. 3rd. edition. Pearson Prentice Hall, Upper Saddle River, NJ.

Johns, B. W. 1977. Golden eagle attempts to kill sandhill crane. Blue Jay 35:92-93.

Johnsgard, P. A. 1983. Cranes of the World: Demoiselle Crane (*Anthropoides virgo*). Papers in the Biological Sciences, University of Nebraska-Lincoln 13:95-102.

Kanwisher, J. W., T. C. Williams, J. M. Teal, and K. O. Lawson Jr. 1978. Radiotelemetry of heart rates from free-ranging gulls. Auk 95:288-293.

Katzner, T. E., E. A. Bragin, S. T. Knick, and A. T. Smith. 2006. Spatial structure in the diet of imperial eagles *Aquila heliaca* in Kazakhstan. Journal of Avian Biology 37:594-600.

Kellie, A. E. 1975. Methods of steroid analysis. II. Competitive binding. Pages 211-226 *in* H. L. J. Makin, editor. Biochemistry of Steroid Hormones. Blackwell Scientific Publishing, Oxford.

Khan, M. Z., J. Altmann, S. S. Isani, and J. Yu. 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. General and Comparative Endocrinology 128:57-64.

Kitaysky, A. S., E. V. Kitaiskaia, J. C. Wingfield, and J. F. Piatt. 2001. Dietary restriction causes chronic elevation of corticosterone and enhances stress response in red-legged kittiwake chicks. Journal of Comparative Physiology B 171:701-709.

Kitaysky, A. S., J. C. Wingfield, and J. F. Piatt. 1999. Dynamics of food availability, body condition and physiological stress response in breeding Black-legged Kittiwakes. Functional Ecology 13:577-584.

Krapu, G. L., D. A. Brandt, K. L. Jones, and D. H. Johnson. 2011. Geographic distribution of the Mid-Continent Population of sandhill cranes and related management applications Wildlife Monographs 175:1-38.

Ladewig, J. 2000. Chronic intermittent stress: a model for the study of long-term stressors. Pages 159-169 *in* G. P. Moberg and J. A. Mench, editors. The biology of animal stress. CABI Publishing, New York, New York, USA.

Layne, J. 1981. Nesting, development of the young, and parental behavior of a pair of Florida Sandhill Cranes. Florida Field Naturalist 9:51-75.

Le Maho, Y., H. Karmann, D. Briot, Y. Handrich, J. P. Robin, E. Mioskowski, Y. Cherel, and J. Farni. 1992. Stress in birds due to routine handling and a technique to avoid it. American Journal of Physiology 263:R775-781.

Leopold, A. S. 1965. Fauna silvestre de Mexico: Aves y mamiferos de caza. Instituto Mexicono de Recursos Naturales Renovables, Mexico, D.F.

Li, B. T., R. J. Christopherson, and S. J. Cosgrove. 2000. Effect of water restriction and environmnental temperatures on metabolic rate and physiological parameters in sheep. Canadian Journal of Animal Science 80:97-104.

Li, C., and W. H. Wong. 2001. Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. Proceedings of the National Academy of Sciences 98:31-36.

Linhart, S. B., and F. F. Knowlton. 1975. Determining the relative abundance of coyotes by scent station lines. Wildlife Society Bulletin 3:119-124.

Littlefield, C. D. 1986. Autumn sandhill crane habitat use in southeast Oregon. The Wilson Bulletin 98:131-137.

Littlefield, C. D., and S. M. Lindstedt. 1992. Survival of juvenile greater sandhill cranes at Malheur National Wildlife Refuge, Oregon. Pages 21-31 *in* 1988 North American Crane Workshop. Florida Nongame Wildlife Program, Gainesville, Florida.

Lopez-Saut, E. G., F. Chavez-Ramirez, and R. Rodriguez-Estrella. 2011. New records of wintering grounds for sandhill cranes in Mexico. Waterbirds 34:239-246.

Lovvorn, J. R., and C. M. Kirkpatrick. 1981. Roosting behavior and habitat of migrant Greater Sandhill Cranes. Journal of Wildlife Management 45:842-857.

Lowe, T. E., N. G. Gregory, A. D. Fisher, and S. R. Payne. 2002. The effects of temperature elevation and water deprivation on lamb physiology, welfare, and meat quality. Australian Journal of Agricultural Research 53:707-714.

Lucas, J. R., T. M. Freeberg, J. Egbert, and H. Schwabl. 2006. Fecal corticosterone, body mass, and caching rates of Carolina chickadees (*Poecile carolinensis*) from disturbed and undisturbed sites. Hormones and Behavior 49:634-643.

Ludders, J. W., J. A. Langenberg, N. M. Czekala, and H. N. Erb. 2001. Fecal corticosterone reflects serum corticosterone in Florida sandhill cranes. Journal of Wildlife Diseases 37:646-652.

Lynch, J. W., M. Z. Khan, J. Altmann, M. N. Njahira, and N. Rubenstein. 2003. Concentrations of four fecal steroids in wild baboons: short-term storage conditions and consequences for data interpretation. General and Comparative Endocrinology 132:264-271.

Lynn, S. E., C. W. Breuner, and J. C. Wingfield. 2003. Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. Hormones and Behavior 43:150-157.

MacArthur, R. A., V. Geist, and R. H. Johnston. 1982. Cardiac and behavioral responses of mountain sheep to human disturbance Journal of Wildlife Management 46:351-358.

Matkovics, B. 1972. In vitro transformation of steroids as a substitute of microbial transformation. Steroids Lipids Research 3:1-7.

McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43:2-15.

McGrew, J. C. 1979. *Vulpes macrotis*. Mammalian Species 123:1-6.

Miller, M. W., N. T. Hobbs, and M. C. Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): reliability of cortisol concentrations in urine and feces. Canadian Journal of Zoology 69:15-24.

Millspaugh, J. J., and B. E. Washburn. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138:189-199.

Millspaugh, J. J., R. J. Woods, K. E. Hunt, K. J. Raedeke, G. C. Brundige, B. E. Washburn, and S. K. Wasser. 2001. Fecal glucocorticoid assays and the physiological stress response in elk. Wildlife Society Bulletin 29:899-907.

Moberg, G. P. 2000. Biological response to stress: implications for animal welfare. Pages 1-21 *in* G. P. Moberg and J. A. Mench, editors. The biology of animal stress. CABI Publishing.

Moen, A. N., S. Whittemore, and B. Buxton. 1982. Effects of disturbance by snowmobiles on heart rate of captive white-tailed deer. New York Fish and Game Journal 29:176-183.

Monroy-Vilchis, O., and A. Velázquez. 2002. Distribución regional y abundancia del lince (*Lynx rufus escuinape*) y el coyote (*Canis latrans cagottis*) por medio de estaciones olfativas: un enfoque espacial. Ciencias Naturales y Agropecuarias 9:293-300.

Mostl, E., S. Messmann, E. Bagu, C. Robia, and R. Palme. 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. Journal of Veterinary Medicine A 46:621-631.

Mostl, E., S. Rettenbacher, and R. Palme. 2005. Measurement of corticosterone metabolites in birds' droppings: An analytical approach. Annals of the New York Academy of Sciences 1046:17-34.

Muñoz-Pulido, R., J. C. Alonso, and J. A. Alonso. 1993. Common crane (*Grus grus*) killed by golden eagle (*Aquila chrysaetos*) Die Vogelwarte 37:78-79.

Nesbitt, S. A., and L. C. Badger. 1995. Coyote preys on young Florida Sandhill Crane. Florida Field Naturalist 23:15-16.

Olsen, G. H. 2004. Mortality of Mississippi Sandhill Crane chicks. Journal of Avian Medicine and Surgery 18:269-272.

Olson, D. M., and E. Dinerstein. 1998. The Global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions. Conservation Biology 12:502-515.

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr, and E. Mostl. 2005. Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion,

and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 1040:162-171.

Pereira, R. J. G., J. M. B. Duarte, and J. A. Negrao. 2006. Effects of environmental conditions, human activity, reproduction, antler cycle and grouping on fecal glucocorticoids of free-ranging Pampas deer stags (*Ozotoceros bezoarticus bezoarticus*). Hormones and Behavior 49:114-122.

Perez-Arteaga, A., S. F. Jackson, E. Carrera, and K. J. Gaston. 2005. Priority sites for wildfowl conservation in Mexico. Animal Conservation 8:41-50.

Pravosudov, V. V., A. S. Kitaysky, C. Saldanha, J. C. Wingfield, and N. S. Clayton. 2001. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees (*Poecile gambeli*). General and Comparative Endocrinology 126:242-248.

Quillfeldt, P., and E. Mostl. 2003. Resource allocation in Wilson's storm-petrels *Oceanites oceanicus* detemined by measurement of glucocorticoid excretion. Acta Ethologica 5:115-122.

R Development Core Team. 2011. R : A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ringer, R. K. 1976. Adrenals. Pages 372-382 *in* P. D. Sturkie, editor. Avian physiology. Springer-Verlag, New York, NY.

Romero, L. M. 2004. Physiological stress in ecology: lessons from biomedical research. Trends in Ecology and Evolution 19:249-255.

Romero, L. M., M. Ramenofsky, and J. C. Wingfield. 1997. Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). comparative Biochemistry and Physiology C 116:171-177.

Romero, L. M., J. M. Reed, and J. C. Wingfield. 2000. Effects of weather on corticosterone responses in wild free-living passerine birds. General and Comparative Endocrinology 118:113-122.

Rushen, J. 2000. Some issues in the interpretation of behavioural responses to stress. Pages 23-42 *in* G. P. Moberg, Mench, J.A., editor. The Biology of Animal Stress. CABI Publishing, New York, USA.

Saltz, D., and G. C. White. 1991. Urinary cortisol and urea nitrogen responses to winter stress in mule deer. Journal of Wildlife Management 55:1-16.

Sapolsky, R. M. 1986. Endocrine and behavioral correlates of drought in wild olive baboons (*Papio anubis*). American Journal of Primatology 11:217-227.

Sapolsky, R. M., L. M. Romero, and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine Reviews 21:55-89.

Sharp, D. E., and W. O. Vogel. 1991. Population status, hunting regulations, hunting activity, and harvest of mid-continent sandhill cranes. Proceeding of the North American Crane Workshop 6:24-32.

Sheffield, C. 1985. Selecting band combinations from multispectral data. Photogrammetic Engineering and Remote Sensing 51:681-687.

Shideler, S. E., A. M. Ortuño, F. M. Moran, E. A. Moorman, and B. L. Lasley. 1993. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. Biology of Reproduction 48:1290-1298.

Siegel, H. S. 1995. Stress, strains and resistance. British Poultry Science 36:3-22.

Silverin, B. 1998. Stress responses in birds. Avian and poultry biology reviews 9:153-168.

SIMBAD. 2012. Sistema Estatal y Municipal de Bases de Datos. INEGI. Mexico. [http://sc.inegi.org.mx/sistemas/cobdem].

Sparling, D. W., and G. L. Krapu. 1994. Communal roosting and foraging behavior of staging sandhill cranes. The Wilson Bulletin 106:62-77.

Stalmaster, M. V., and J. L. Kaiser. 1997. Flushing responses of wintering bald eagles to military activity. Journal of Wildlife Management 61:1307-1313.

Stenseth, N. C., H. Leirs, A. Skonhoft, S. A. Davis, R. P. Pech, H. P. Andreassen, G. R. Singleton, M. Lima, R. S. Machang'u, R. H. Makundi, Z. Zhang, P. R. Brown, D. Shi,

and X. Wan. 2003. Mice, rats, and people: the bio-economics of agricultural rodent pests. Frontiers in Ecology and the Environment 1:367-375.

Stoleson, S. H., R. S. Felger, G. Ceballos, C. Raish, M. F. Wilson, and A. Burquez. 2005. Recent history of natural resource use and population growth in northen Mexico. Pages 52-86 *in* J. E. Cartron, G. Ceballos, and R. S. Felger, editors. Biodiversity, ecosystems, and conservation in northern Mexico. Oxford Universtiy Press, Inc., New York.

Sutherland, W. J., and R. E. Green. 2004. Habitat assessment. Pages 251-269 *in* W. J. Sutherland, I. Newton, and R. E. Green, editors. Bird Ecology and Conservation: A Handbook of Techniques. Oxford University Press, Oxford, UK.

Tacha, T. C. 1988. Social organization of sandhill cranes from midcontinental North America. Wildlife Monographs 99:3-37.

Tempel, D. J., and R. J. Gutierrez. 2003. Fecal corticosterone levels in California spotted owls exposed to low-intensity chainsaw sound. Wildlife Society Bulletin 31:698-702.

Terio, K. A., J. L. Brown, R. Moreland, and L. Munson. 2002. Comparison of different drying and storage methods on quantifiable concentrations of fecal steroids in the cheetah. Zoo Biology 21:215-222.

Thaker, M., S. L. Lima, and D. K. Hews. 2009. Acute corticosterone elevation enhances antipredator behaviors in male tree lizard morphs. Hormones and Behavior 56:51-57.

Thiollay, J. M. 1979. La migration des grues a travers l'Himalaya et la predtion par les aigles royaux. Alauda 47:83-92.

Thomas, L., S. T. Buckland, E. A. Rexstad, J. L. Laake, S. Strindberg, S. L. Hedley, J. R. B. Bishop, T. A. Marques, and K. P. Burnham. 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology 47:5-14.

Tjernberg, M. 1981. Diet of the golden eagle Aquila chrysaetos during the breeding season in Sweden. Holarctic Ecology 4:12-19.

Touma, C., N. Sachser, E. Mostl, and R. Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology 130:267-278.

Verme, L. J., and R. V. Doepker. 1988. Suppression of reproduction in Upper Michigan white-tailed deer, *Odocoileus virginianus*, by climatic stress during the rut. Canadian Field-Naturalist 102:550-552.

Walkinshaw, L. H. 1949. The sandhill cranes. Cranbrook Institute of Science, Bloomfield Hills, Michigan.

Washburn, B. E., and J. J. Millspaugh. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. General and Comparative Endocrinology 127:217-222.

Washburn, B. E., J. J. Millspaugh, J. H. Schulz, S. B. Jones, and T. Mong. 2003. Using fecal glucocorticoids for stress assessment in mourning doves. The Condor 105:696-706.

Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbance in the Northern Spotted Owl (*Strix occidentalis caurina*). Conservation Biology 11:1019-1022.

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

Wasser, S. K., S. Papageorge, C. Foley, and J. L. Brown. 1996. Excretory fate of estradiol and progesterone in the African elephant (*Loxodonta africana*) and patterns of fecal steroid concentrations throughout the estrous cycle. General and Comparative Endocrinology 102:255-262.

Watson, J. 1997. The Golden Eagle. T. & A.D. Poyser, London.

Weingrill, T., D. A. Gray, L. Barrett, and S. P. Henzi. 2004. Fecal cortisol levels in free-ranging female chacma baboons: relationship to dominance, reproductive state and environmental factors. Hormones and Behavior 45:259-269.

White, C. M., and T. L. Thurow. 1985. Reproduction of ferruginous hawks exposed to controlled disturbance. Condor 87:14-22.

Wilson, G. J., and R. J. Delahay. 2001. A review of methods to estimate the abundance of terrestrial carnivores using field signs and observation Wildlife Research 28:151-164.

Windingstad, R. M., H. E. Stiles, and R. C. Drewien. 1981. Whooping crane preyed upon a golden eagle. The Auk 98:393-394.

Wingfield, J. C. 1988. Changes in reproductive function of free-living birds in direct response to environmental perturbations. Pages 121-148 *in* M. H. Stetson, editor. Processing of environmental information in vertebrates. Springer, Berlin, Germany.

Wingfield, J. C., P. Deviche, S. Sharbaugh, L. B. Astheimer, R. Holberton, R. Suydam, and K. Hunt. 1994a. Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flamea*, in Alaska. Journal of Experimental Zoology 270:372-380.

Wingfield, J. C., D. L. Maney, C. W. Breuner, J. D. Jacobs, S. Lynn, M. Ramenofsky, and R. D. Richardson. 1998. Ecological bases of hormone-behavior interactions: the "emergency life history stage. American Zoologist 38:191-206.

Wingfield, J. C., and M. Ramenofsky. 1999. Hormones and the behavioral ecology of stress. Pages 1-51 *in* P. H. M. Balm, editor. Stress physiology in animals. Sheffield Academic, Sheffield, UK.

Wingfield, J. C., R. Suydam, and K. Hunt. 1994b. The adrenocortical responses to stress in snow buntings (*Plectrophenax nivalis*) and Lapland longspurs (*Calcarius lapponicus*) at Barrow, Alaska. Comparative Biochemistry and Physiology B 108:299-306.

Wingfield, J. C., C. M. Vleck, and M. C. Moore. 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. The Journal of Experimental Zoology 264:419-428.

Woods, G. F. 1975. Chemical and microbiological transformation of steroids. Pages 5-10 *in* E. H. D. Cameron, S. G. Hillier, and K. Griffiths, editors. Steroid Immunoassay. Alpha Omega, Cardiff, UK.

Zuur, A. F., E. N. Ieno, and G. M. Smith. 2007. Analysing ecological data. Springer, New York, NY.

Table 2.1. Geographic location of the wetlands included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Number | Wetland | Location | State | Municipality |
|---|---|---|---|---|
| 1 | Laguna de Ojo Federico | 31°2’57.21’’N 107°55’1.46’’W | Chihuahua | Ascensión |
| 2 | Laguna de Babícora | 29°21’46.64’’N 107°47’15.68’’W | Chihuahua | Gómez Farías |
| 3 | Laguna de Mexicanos | 28°10’36.44’’N 106°55’42.09’’W | Chihuahua | Cusihuiriachi |
| 4 | Presa San Carlos de Mapimí | 26°34’2.11’’N 103°44’49.56’’W | Durango | Tlahualilo |
| 5 | Laguna de Santiaguillo | 24°54’38.83’’N 104°52’27.28’’W | Durango | Nuevo Ideal |
| 6 | Laguna de San Juan de Ahorcados | 24°1’21.18’’N 102°17’48.14’’W | Zacatecas | General Francisco R. Murguía |

Table 2.2. Characteristics of satellite images downloaded from the Earth Science Data Interface (ESDI) used to estimate resource availability surrounding six wetlands included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Location | ID[a] | WRS: P/R[b] | Date Acquired | Sensor[c] | Producer[d] | Attributes[e] | Type[f] | Coordinate System[g] |
|---|---|---|---|---|---|---|---|---|
| Ojo Federico | 216-450 | 2: 034/038 | 10-21-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone12N |
| | 216-451 | 2: 034/039 | 10-21-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone12N |
| Babícora | 216-419 | 2: 033/040 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| Mexicanos | 216-419 | 2: 033/040 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| | 216-399 | 2: 032/040 | 10-10-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| | 216-420 | 2: 033/041 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| Santiaguillo | 216-371 | 2: 031/043 | 10-19-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| | 216-345 | 2: 030/043 | 11-10-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| San Carlos de Mapimí | 216-344 | 2: 030/042 | 10-28-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| San Juan de Ahorcados | 216-320 | 2: 029/043 | 10-18-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |

[a] Landsat imagery online identification number.

[b] WRS = Worldwide Reference System, P = Path, R = Row.

[c] ETM+ = Enhanced Thematic Mapper Plus (provided by Landsat 7).

[d] USGS = United States Geological Survey.

[e] Ortho = Orthorectified, GLS2005 = Global Land Survey 2005.

[f] GeoTIFF = Geographic Tagged Image File Format.

[g] WGS_84 = World Geodetic System 1984, UTM = Universal Transverse Mercator.

Table 2.3. Wetland characteristics obtained from spectral and spatial analyses of satellite images to estimate area of agricultural land surrounding each wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Wetland | State | Cropland Area ($km^2$) | Foraging Area ($km^2$) | Proportion Cropland |
|---|---|---|---|---|
| Laguna de Mexicanos | Chihuahua | 664.5 | 1,867.3 | 0.356 |
| Laguna de Santiaguillo | Durango | 1,292.1 | 3,722.2 | 0.347 |
| Laguna de Babícora | Chihuahua | 800.6 | 2,788.4 | 0.287 |
| Laguna de Ojo Federico | Chihuahua | 239.2 | 1,729.5 | 0.138 |
| Laguna de San Juan de Ahorcados | Zacatecas | 102.5 | 1,599.5 | 0.064 |
| Presa San Carlos de Mapimí | Durango | 3.9 | 1,329.7 | 0.003 |

Table 2.4. Wetland characteristics obtained from spectral and spatial analyses of satellite images to estimate the size of each wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Wetland | State | Maximum Area ($km^2$) | Minimum Area ($km^2$) | Average Area ($km^2$) |
|---|---|---|---|---|
| Laguna de Santiaguillo | Durango | 243.1 | 101.8 | 172.5 |
| Laguna de Babícora | Chihuahua | 224.6 | 33.1 | 128.9 |
| Laguna de Mexicanos | Chihuahua | 40.5 | 24.6 | 32.6 |
| Laguna de Ojo Federico | Chihuahua | 30.6 | 10.7 | 20.7 |
| Laguna de San Juan de Ahorcados | Zacatecas | 12.9 | 6.7 | 9.8 |
| Presa San Carlos de Mapimí | Durango | 1.0 | 0.2 | 0.6 |

Table 2.5. Wetland characteristics obtained from spectral and spatial analyses of satellite images to estimate the distance between the center of each wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 and the closest city of population 10,000 or more.

| Wetland | State | Closest City | Population | Distance (km) |
|---|---|---|---|---|
| Presa San Carlos de Mapimí | Durango | Gómez Palacio | 257,352 | 114.9 |
| Laguna de San Juan de Ahorcados | Zacatecas | Río Grande | 32,944 | 78.4 |
| Laguna de Babícora | Chihuahua | Madera | 15,447 | 34.7 |
| Laguna de Mexicanos | Chihuahua | Cuauhtémoc | 114,007 | 29.3 |
| Laguna de Santiaguillo | Durango | Nuevo Ideal | 10,876 | 25.9 |
| Laguna de Ojo Federico | Chihuahua | Ascensión | 13,456 | 9.7 |

Table 2.6. Density of birds of prey at each wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. Data obtained from line transects and analyzed using program Distance.

| Wetland | No. Observations | Per Unit Effort | Density (individuals/ha) | SE | Lower CI | Upper CI | Detection Probability (P) | ESW* (m) |
|---|---|---|---|---|---|---|---|---|
| Laguna de Babícora | 57 | 10.0 | 0.412 | 0.152 | 0.175 | 0.971 | 61.8 | 43.0 |
| Laguna de Mexicanos | 195 | 32.8 | 0.091 | 0.009 | 0.073 | 0.112 | 51.7 | 203.8 |
| Laguna de Santiaguillo | 99 | 46.0 | 0.060 | 0.019 | 0.028 | 0.126 | 11.1 | 112.3 |
| Laguna de San Juan de Ahorcados | 35 | 25.4 | 0.027 | 0.014 | 0.008 | 0.090 | 15.5 | 157.1 |
| Laguna de Ojo Federico | 18 | 17.6 | 0.027 | 0.008 | 0.015 | 0.048 | 97.0 | 118.5 |
| Presa San Carlos de Mapimí | 12 | 25.5 | 0.006 | 0.003 | 0.002 | 0.017 | 47.5 | 229.7 |

* ESW = Effective Strip Width.

Table 2.7. List of best Generalized Linear Mixed Models (GLMM) used to examine the effects of location to the levels of corticosterone from Sandhill Cranes (*Grus canadensis*) wintering in northern Mexico during 2007 and 2009 (see Appendix C for the rest of the models). Location (i.e., wetland site) was a random effect in every model.

| Model | AIC[a] | ΔAIC[b] | $k$[c] | $w_i$[d] |
|---|---|---|---|---|
| LogCort[e] ~ Month + Year + WetS[f] + Month * Year + Month * WetS + Year * WetS | 29.2 | 0.00 | 15 | 1 |
| LogCort ~ Month + Year + CityD[g] + Month * Year + Month * CityD + Year * CityD | 66.7 | 37.5 | 15 | < 0.001 |
| LogCort ~ Month + Year + Month * Year | 79.2 | 50.0 | 10 | < 0.001 |
| LogCort ~ Month + Year | 79.9 | 50.7 | 7 | < 0.001 |
| LogCort ~ Month + Year + WetS | 80.3 | 51.1 | 8 | < 0.001 |
| LogCort ~ Month + Year + WetS + CityD | 82.3 | 53.1 | 9 | < 0.001 |
| LogCort ~ Month + Year + WetS + CityD + BirdsPD[h] | 82.9 | 53.7 | 10 | < 0.001 |
| LogCort ~ Month + FoodA[i] + Month * FoodA | 104.3 | 75.1 | 10 | < 0.001 |
| LogCort ~ Month + NCranes[j] + Month * NCranes | 123.2 | 94.0 | 10 | < 0.001 |
| LogCort ~ Month + WetS + Month * WetS | 132.8 | 103.6 | 10 | < 0.001 |
| Null model | 231.1 | 151.9 | 3 | < 0.001 |

[a] AIC = Akaike's Information Criterion

[b] ΔAIC = Delta Akaike's Information Criterion

[c] $k$ = Number of parameters

[d] $w_i$ = Weight of the model

[e] LogCort = Log transformed corticosterone concentration (ng/g)

[f] WetS = Wetland size

[g] CityD = Distance to city

[h] BirdsPD = Density of birds of prey

[i] FoodA = Food abundance

[j] NCranes = Number of cranes

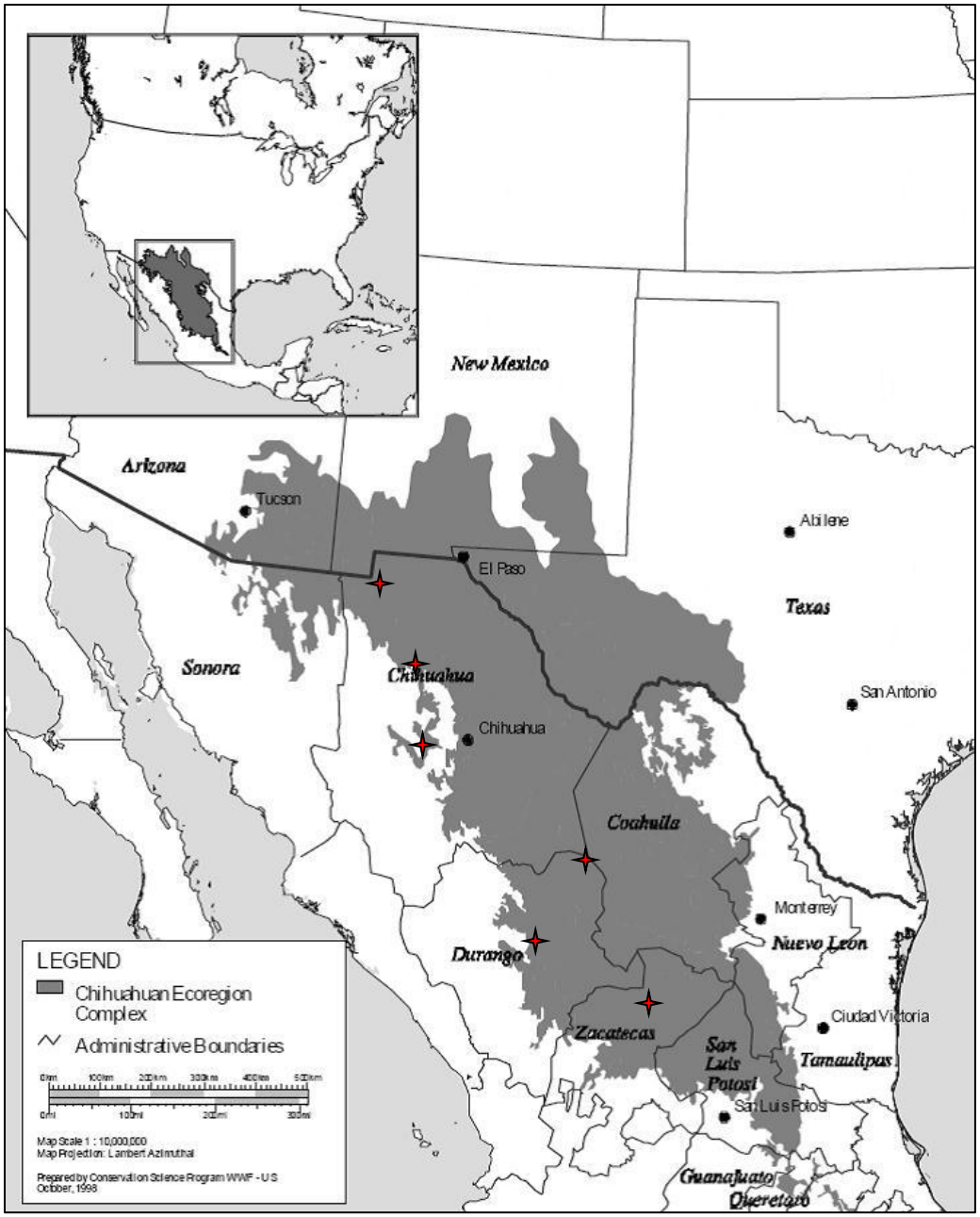



Figure 2.1. Location of the Chihuahuan Desert Ecoregion with the six wetlands selected for a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 the study. Modified from Dinerstein et al. (2001). (Used with permission: Conservation Science Program WWF-US, 1998).

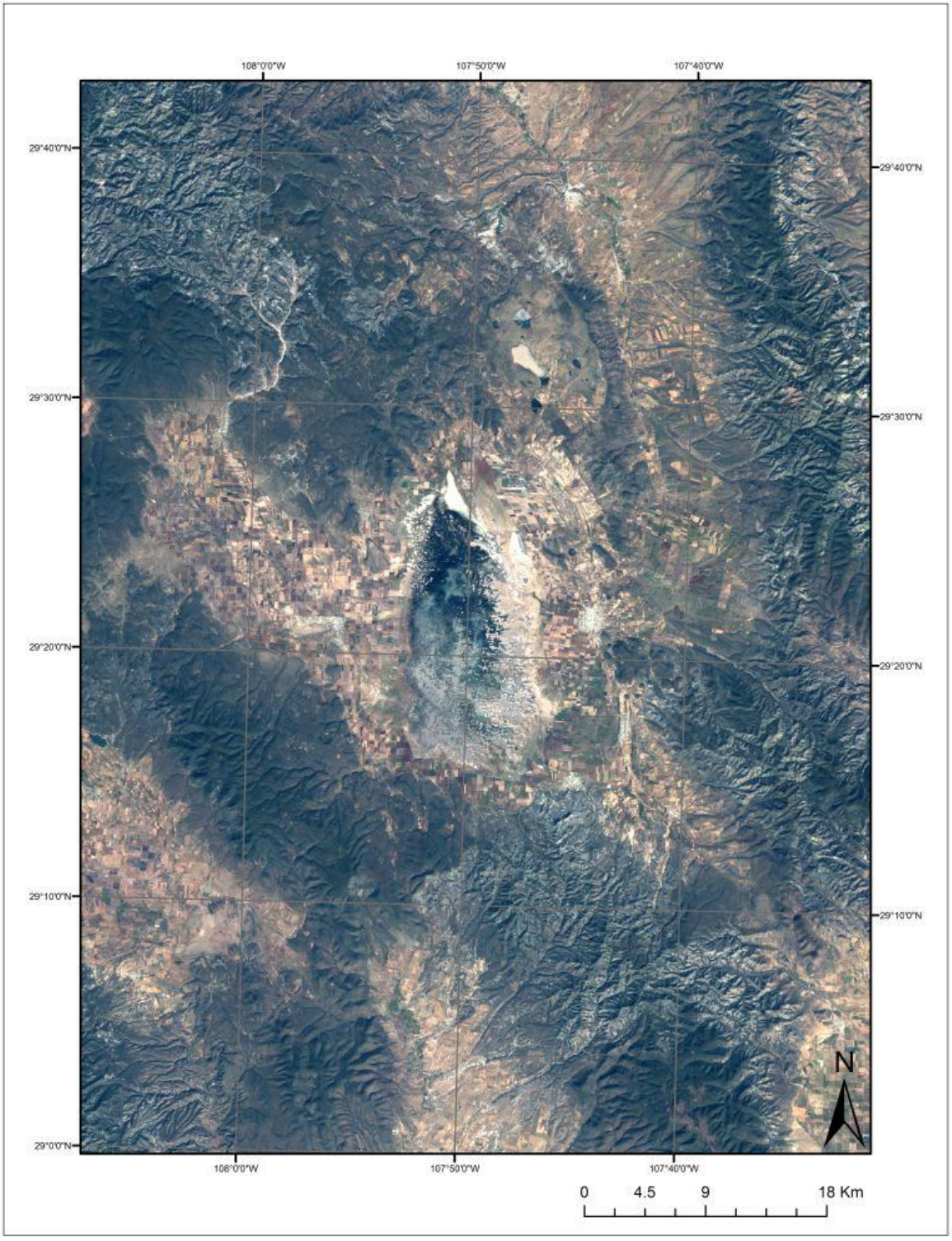



Figure 2.2. Satellite image of the study area located in the Laguna de Babícora, Chihuahua, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.


Figure 2.3. Satellite image of the study area located in the Laguna de Mexicanos, Chihuahua, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.

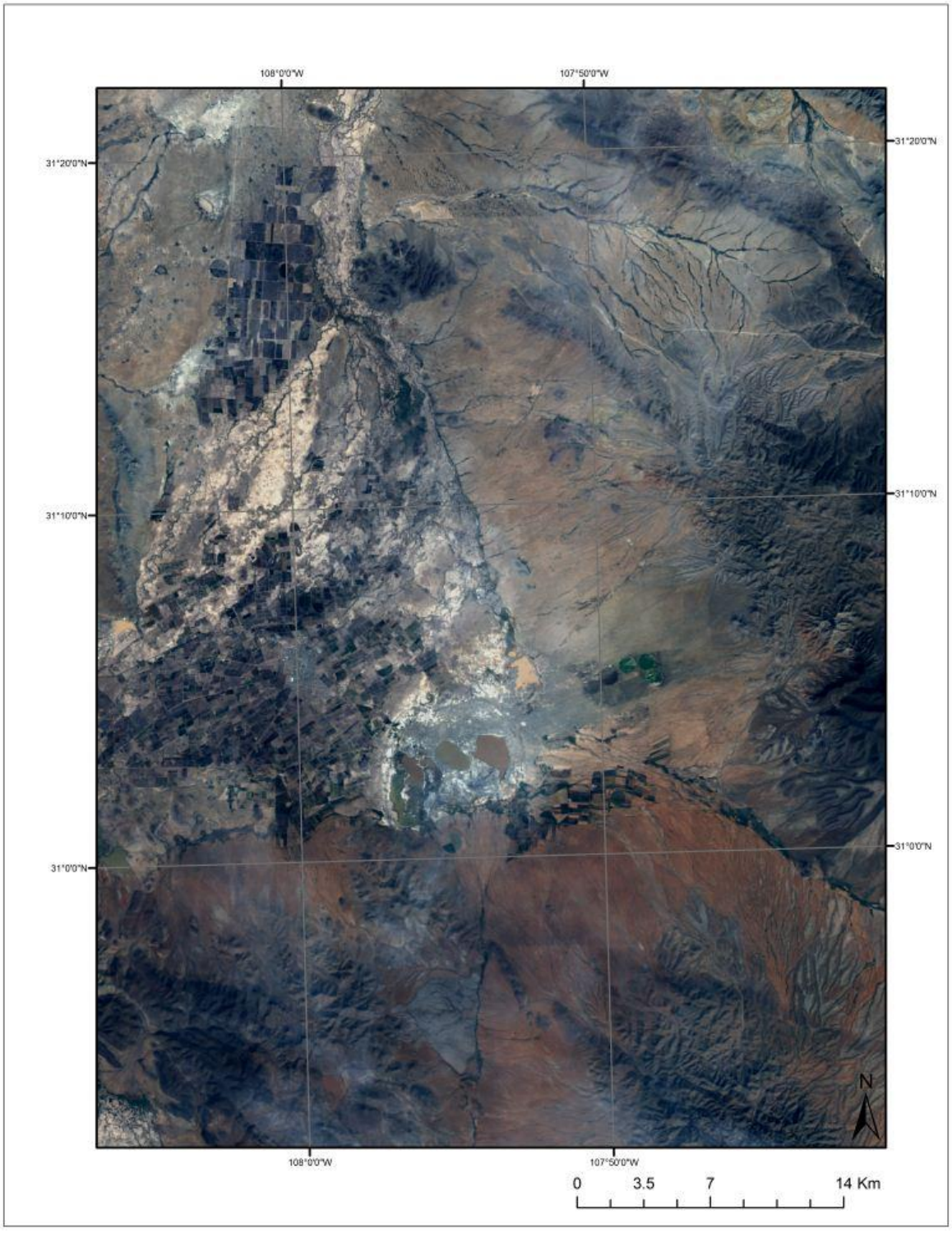



Figure 2.4. Satellite image of the study area located in the Laguna de Ojo Federico, Chihuahua, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.

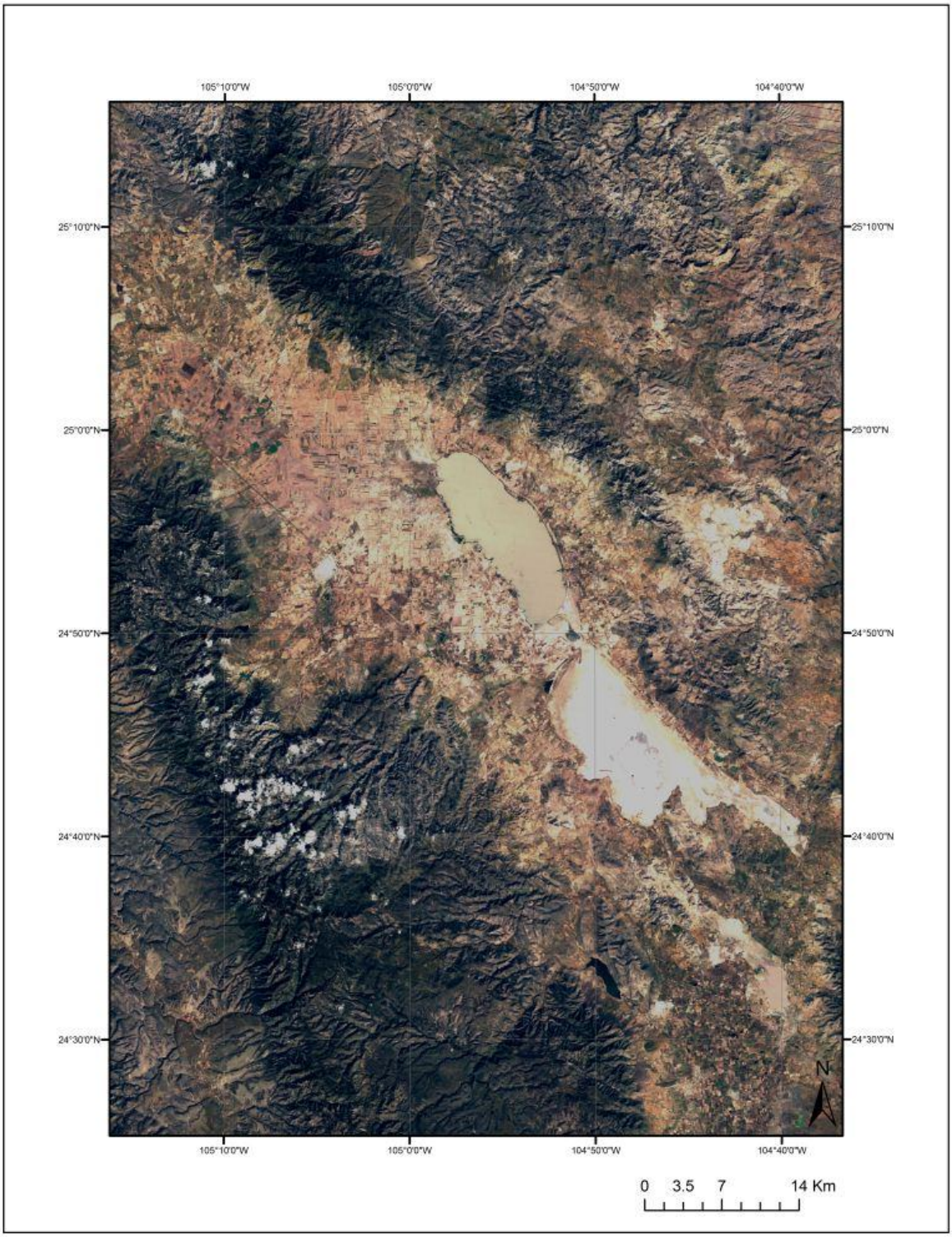



Figure 2.5. Satellite image of the study area located in the Laguna de Santiaguillo, Durango, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.

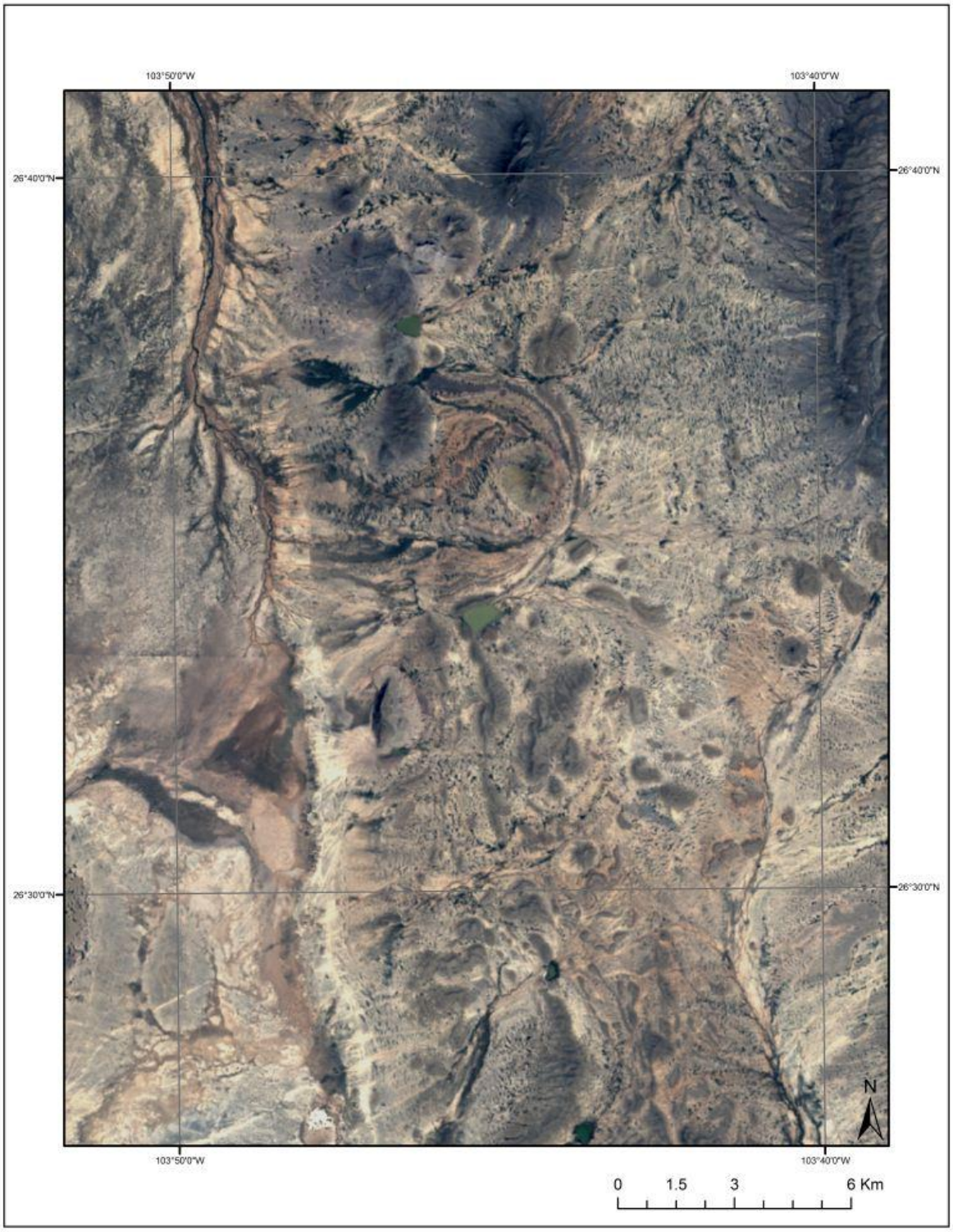



Figure 2.6. Satellite image of the study area located in the Presa San Carlos de Mapimí, Durango, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.


Figure 2.7. Satellite image of the study area located in the Laguna de San Juan de Ahorcados, Zacatecas, Mexico included in a hormonal study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09.

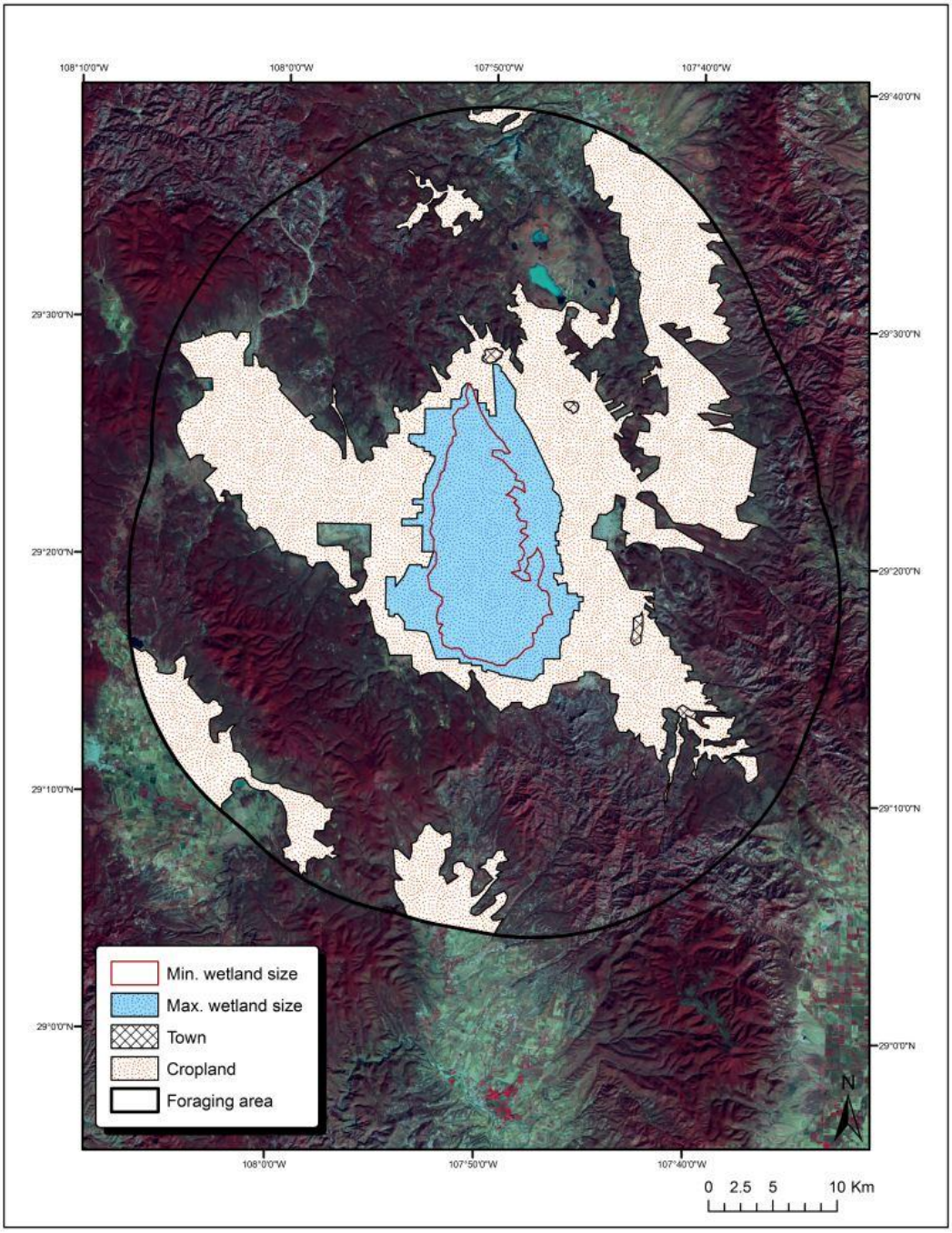



Figure 2.8. Satellite image of the Laguna de Babícora, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

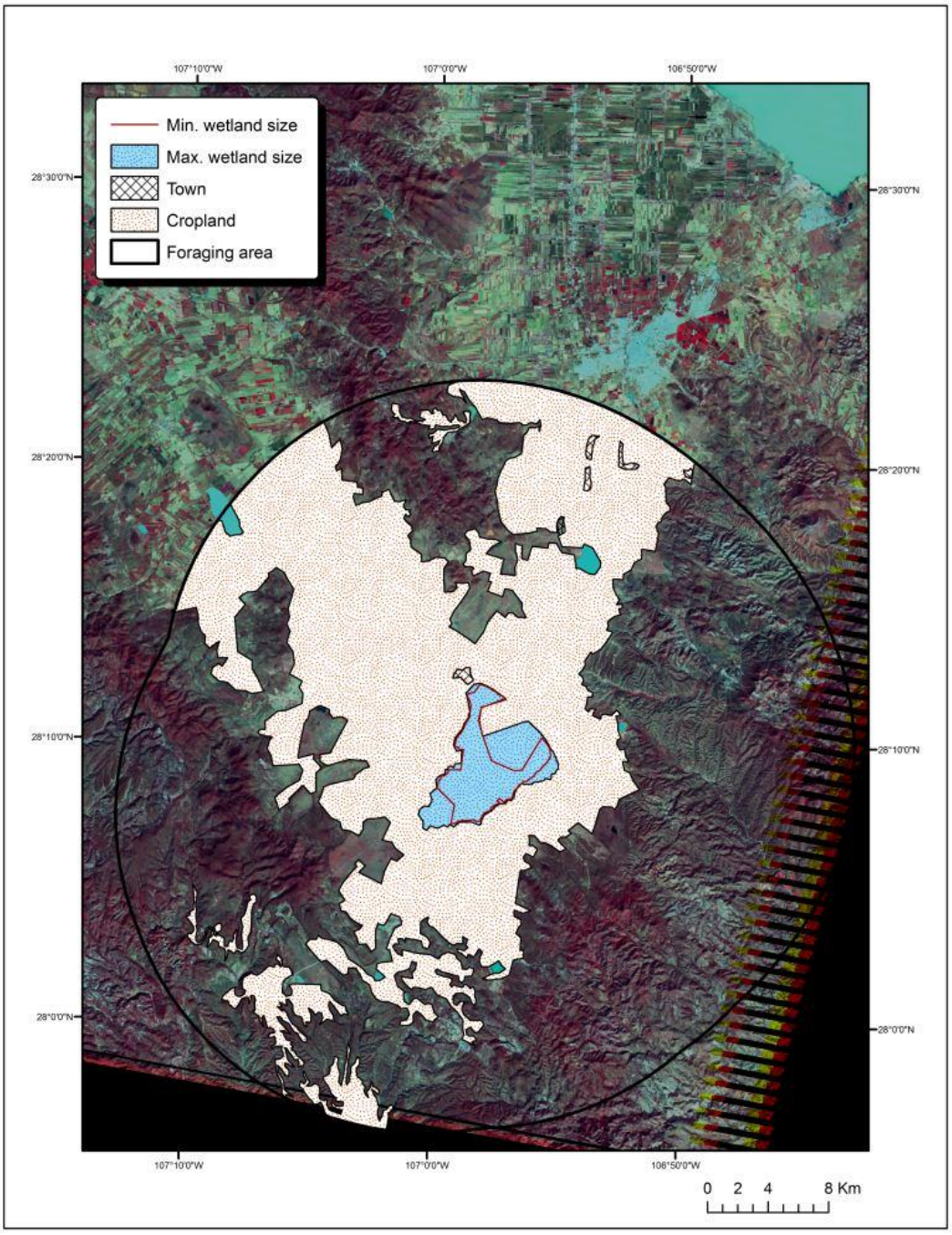



Figure 2.9. Satellite image of the Laguna de Mexicanos, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

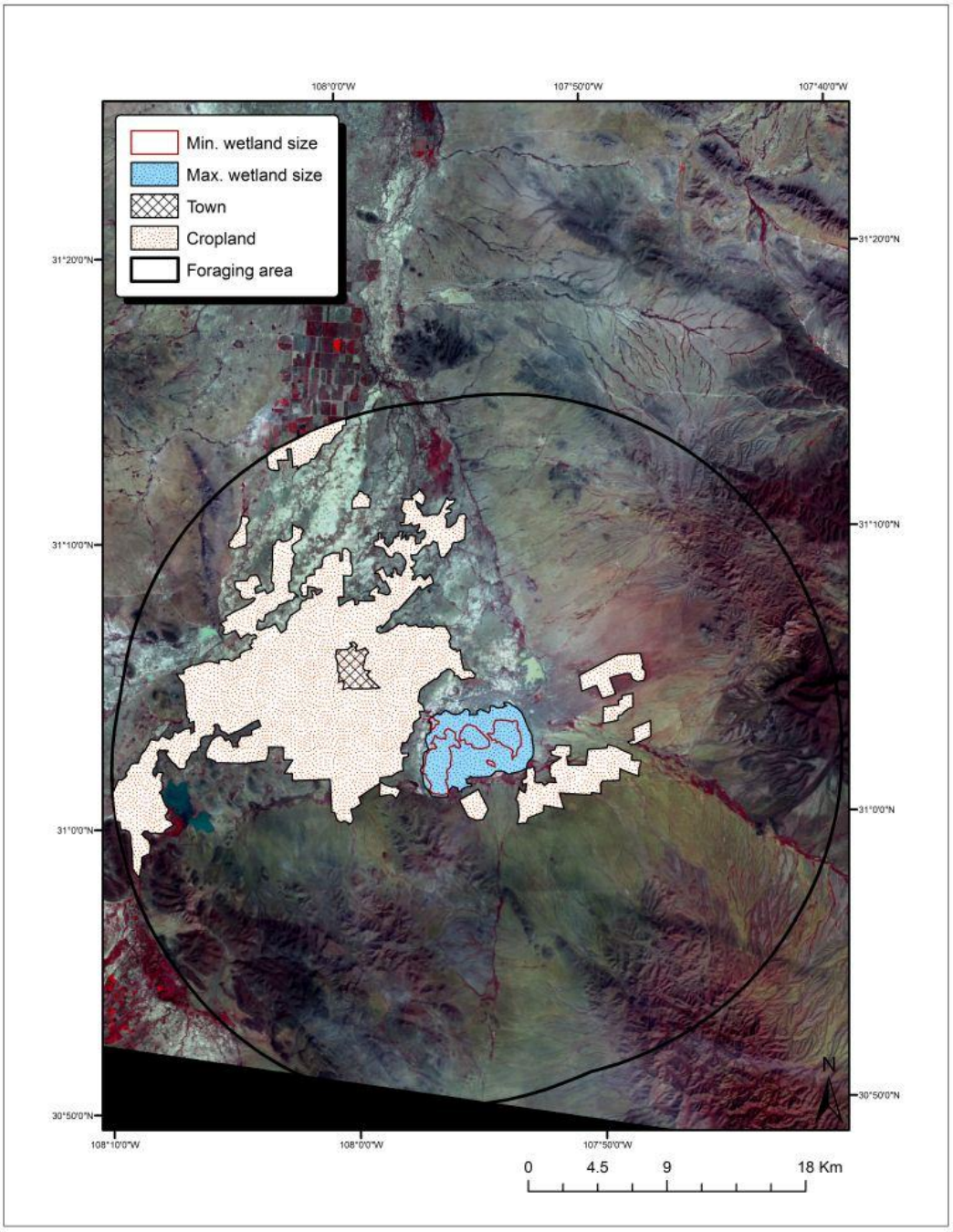



Figure 2.10. Satellite image of the Laguna de Ojo Federico, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

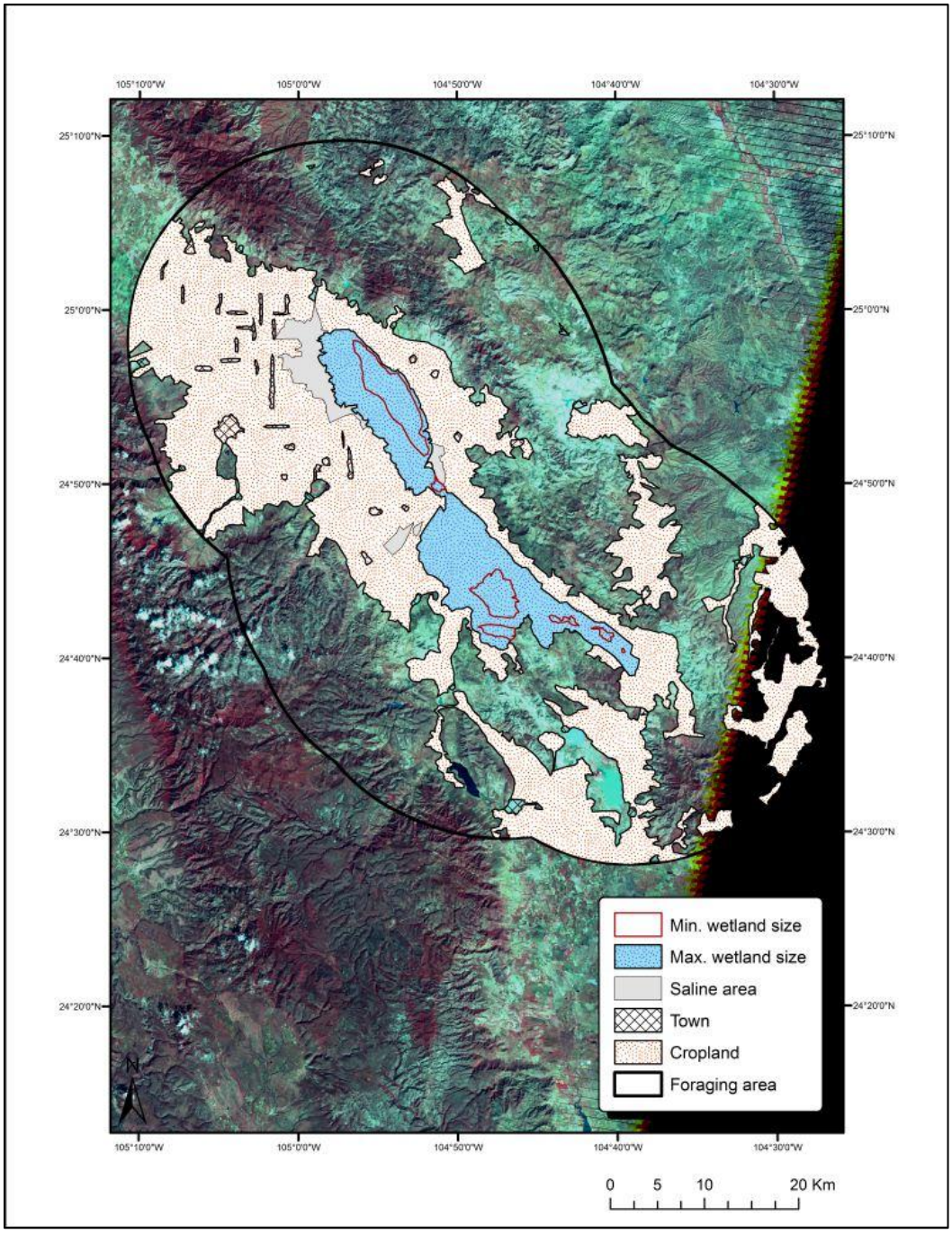



Figure 2.11. Satellite image of the Laguna de Santiaguillo, Durango, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

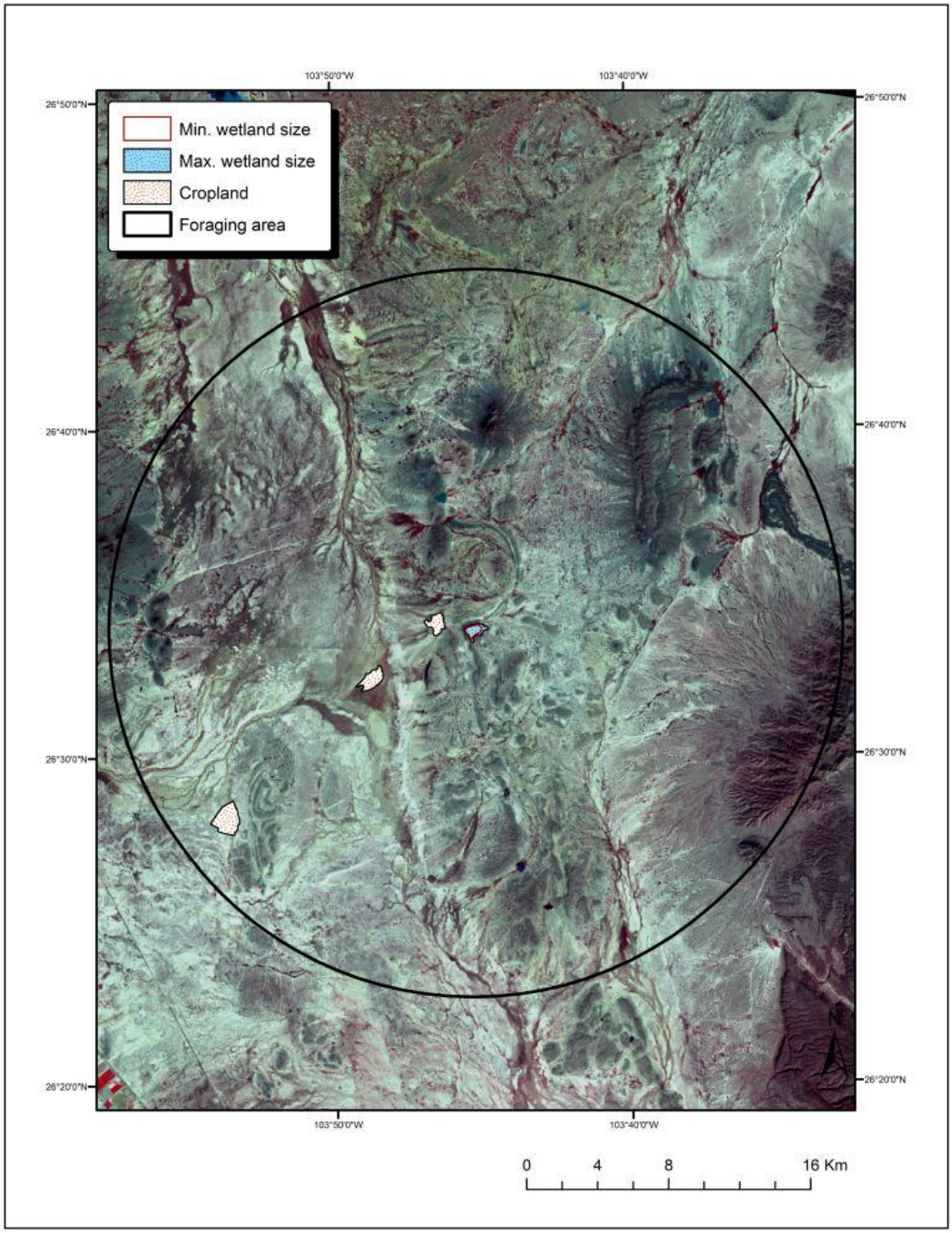



Figure 2.12. Satellite image of the Presa San Carlos de Mapimí, Durango, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

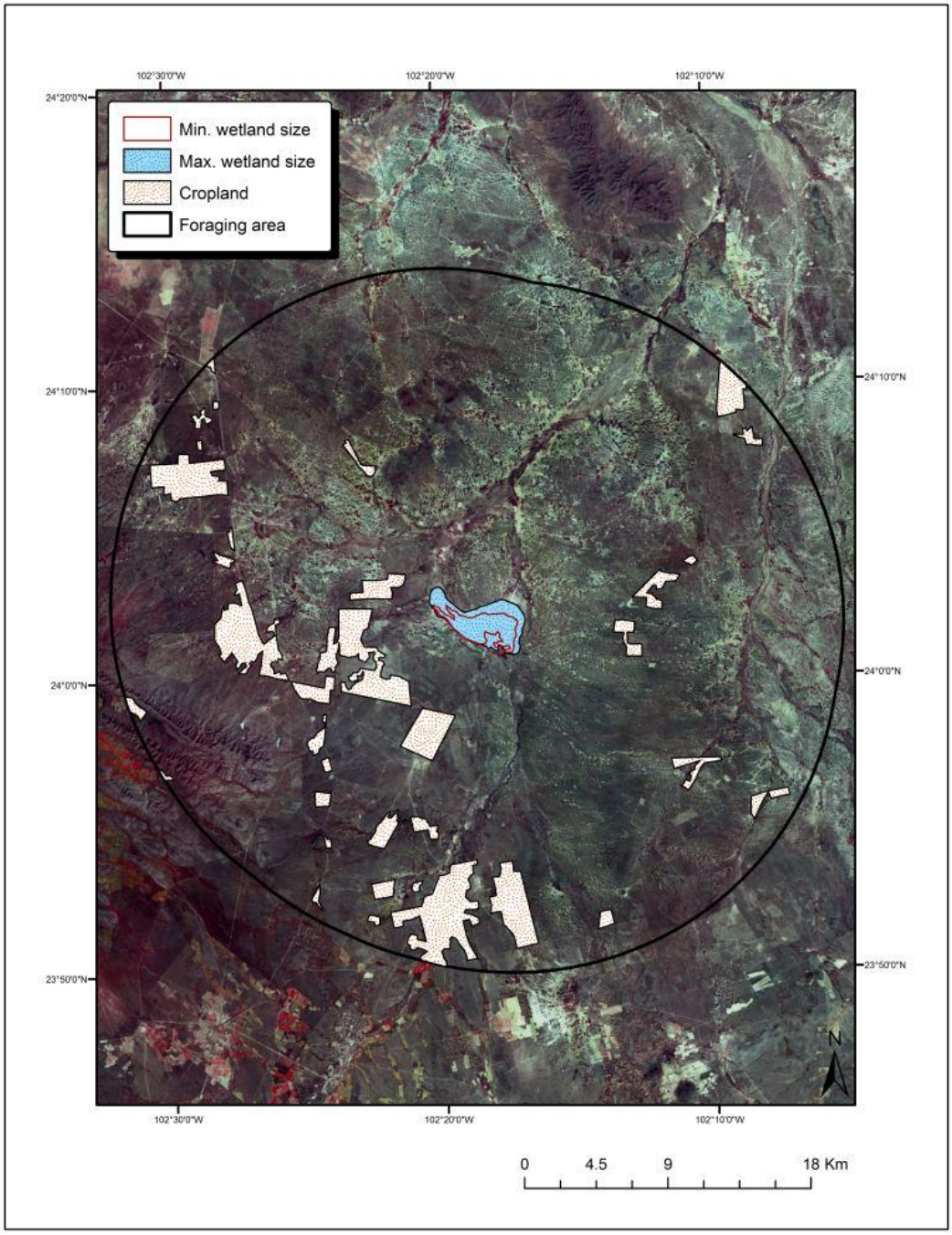



Figure 2.13. Satellite image of the Laguna de San Juan de Ahorcados, Zacatecas, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

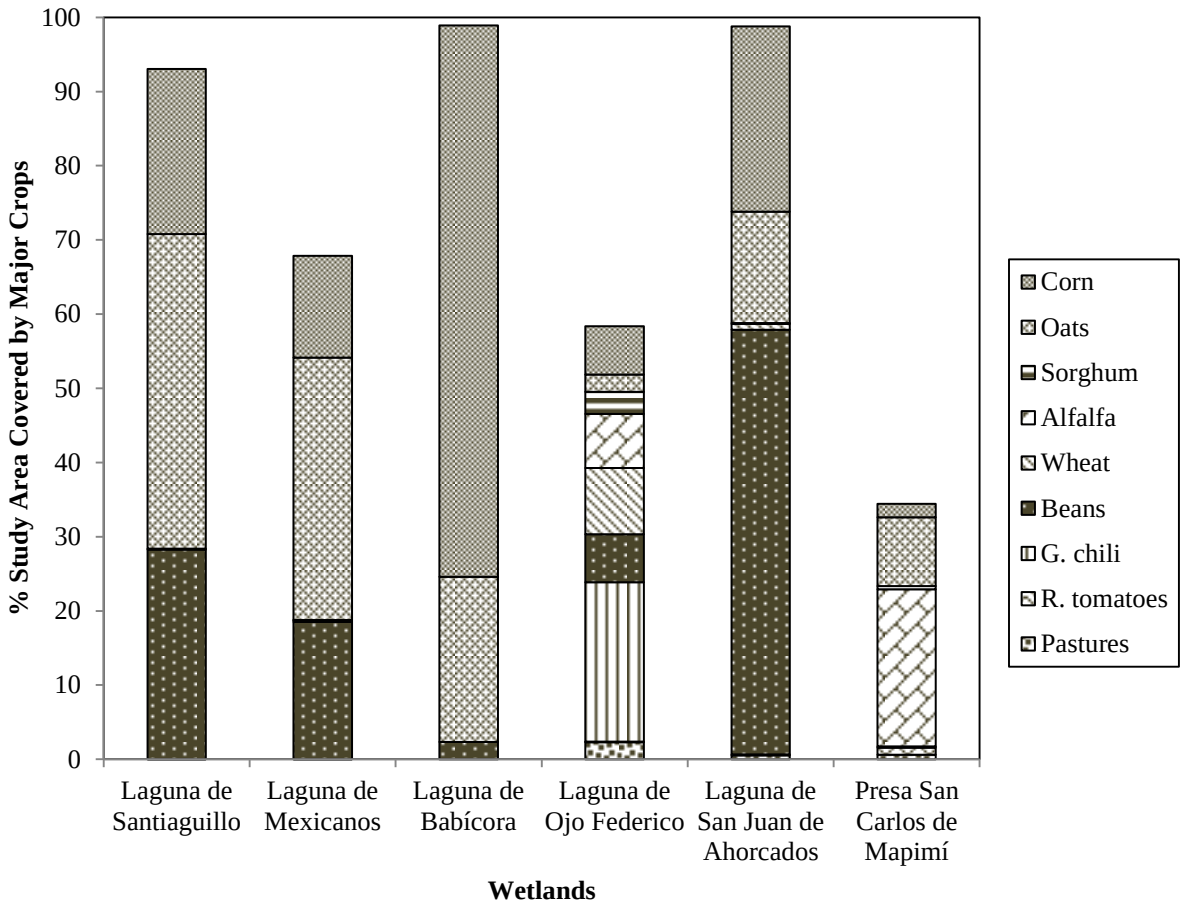
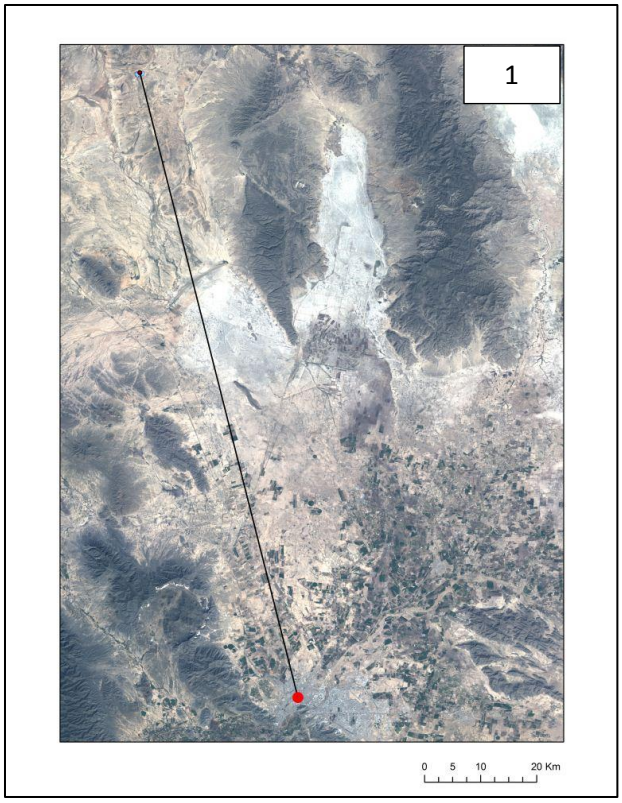



Figure 2.14. Percentage of major crops sown in the municipality where the wetlands in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 were located. Municipalities containing the wetlands were Gómez Farías, Cusihuiriachi, Ascención, Nuevo Ideal, General Francisco R. Murguía, and Tlahualilo. Data reported by the Sistema Estatal y Municipal de Bases de Datos (SIMBAD 2012) and obtained from the Instituto Nacional de Estadística y Geografía (INEGI 2012). The percentage reflects the mean value of the three years while the study was conducted (2007, 2008, and 2009).


1
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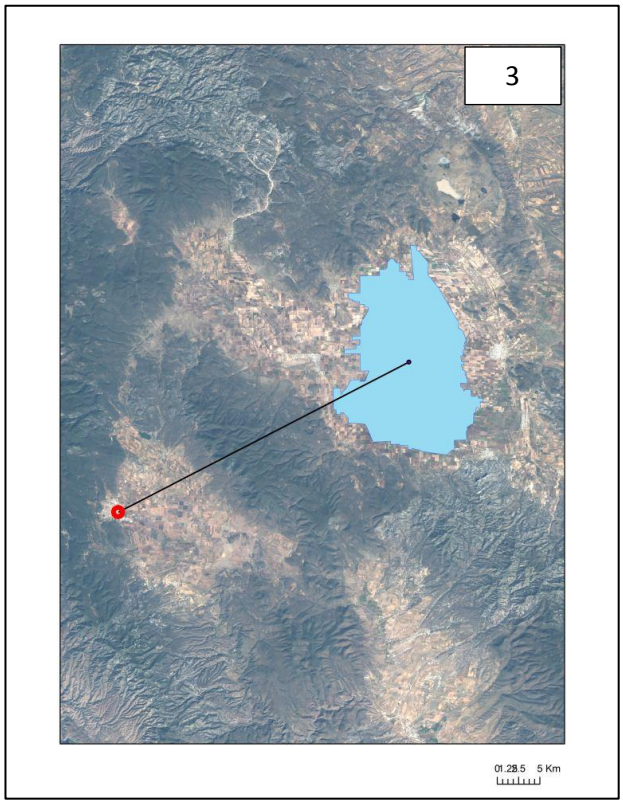
3
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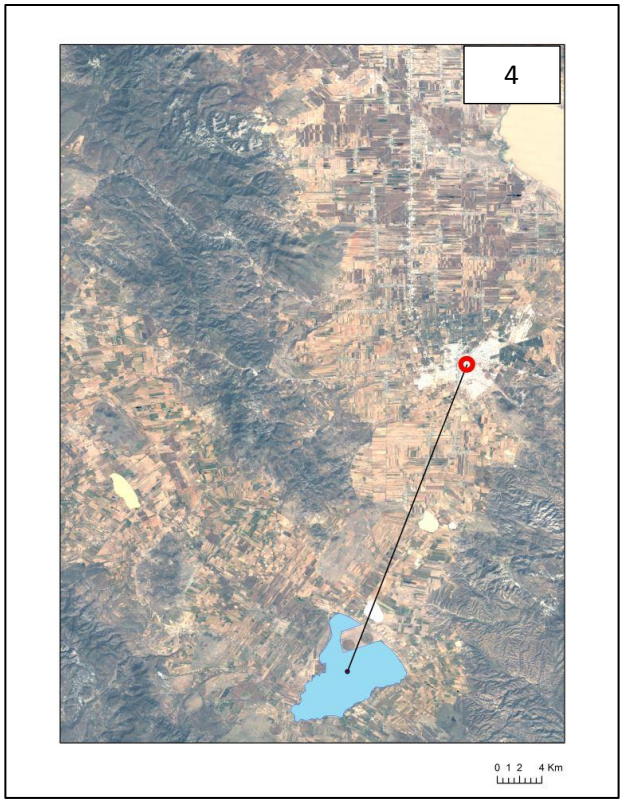
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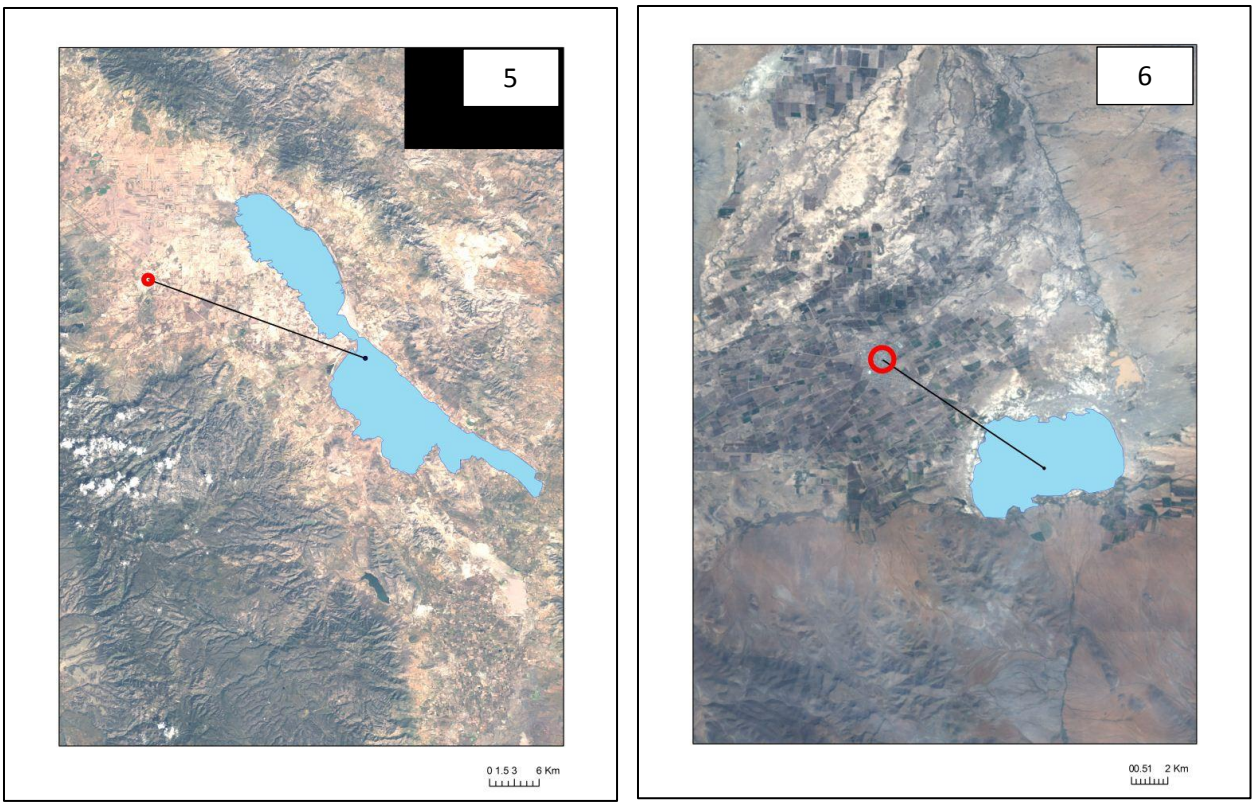


Figure 2.15. Satellite images of the six wetlands included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 indicating the distance between the centroid of each wetland and the closest city of population 10,000 or more: (1) Presa San Carlos de Mapimí to Gómez Palacio; (2) Laguna de San Juan de Ahorcados to Río Grande; (3) Laguna de Babícora to Madear; (4) Laguna de Mexicanos to Cuauhtémoc; (5) Laguna de Santiaguillo to Nuevo Ideal; (6) Laguna de Ojo Federico to Ascensión. Data reported by the Sistema Estatal y Municipal de Bases de Datos (SIMBAD 2012) and obtained from the Censo de Población y Vivienda 2010 from the Instituto Nacional de Estadística y Geografía (INEGI 2012).

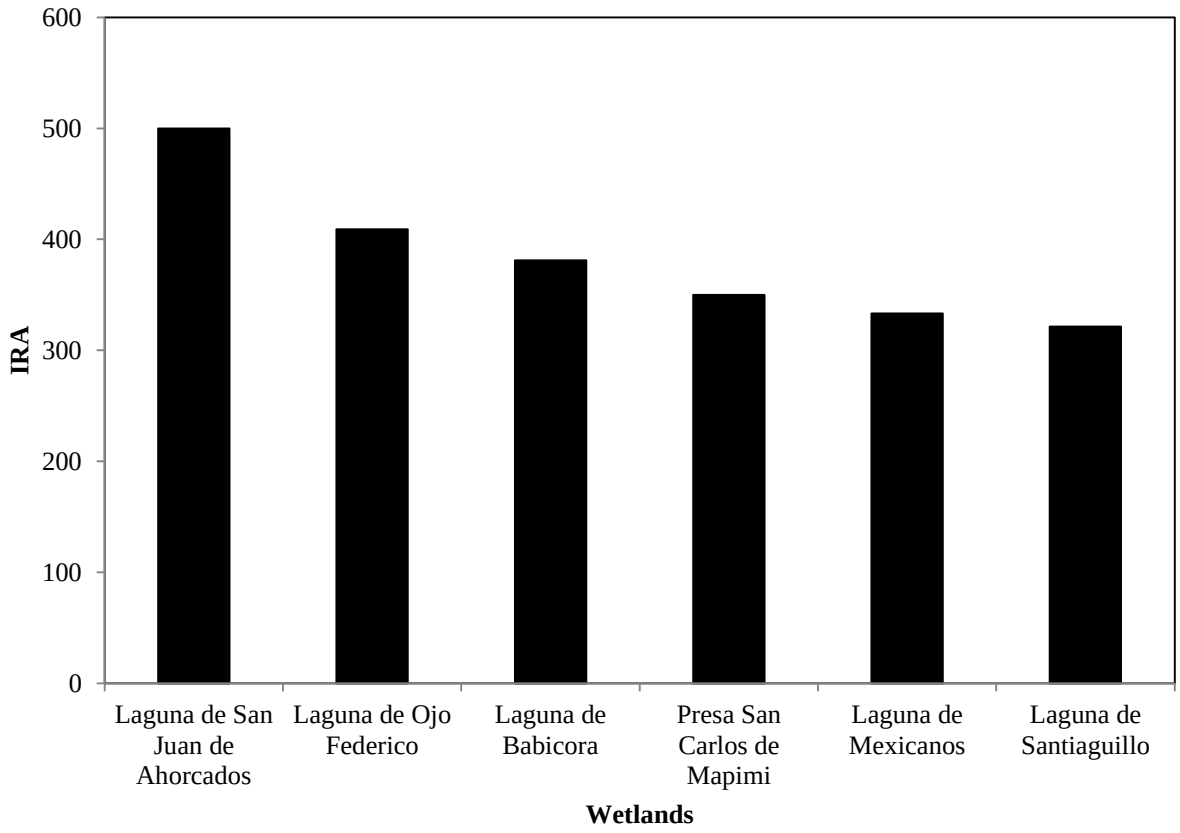


Figure 2.16. Index of relative abundance (IRA) of carnivores obtained from scent station transects along the six wetlands included in a hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

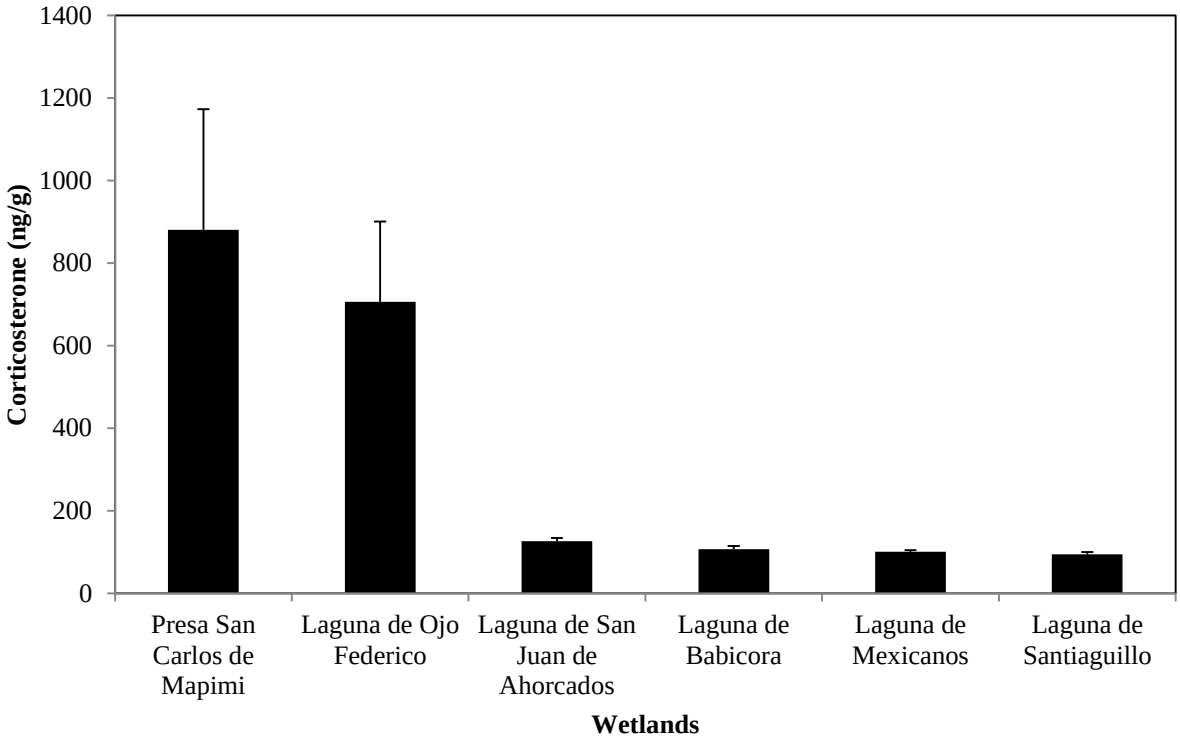


Figure 2.17. Mean fecal corticosterone concentrations (±SE) expressed in ng of hormone per g of dry feces collected in the six wetlands included in the hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico combining both winters (October to February) of 2007/08 and 2008/09. Outliers were removed beyond 3 SD of the mean.

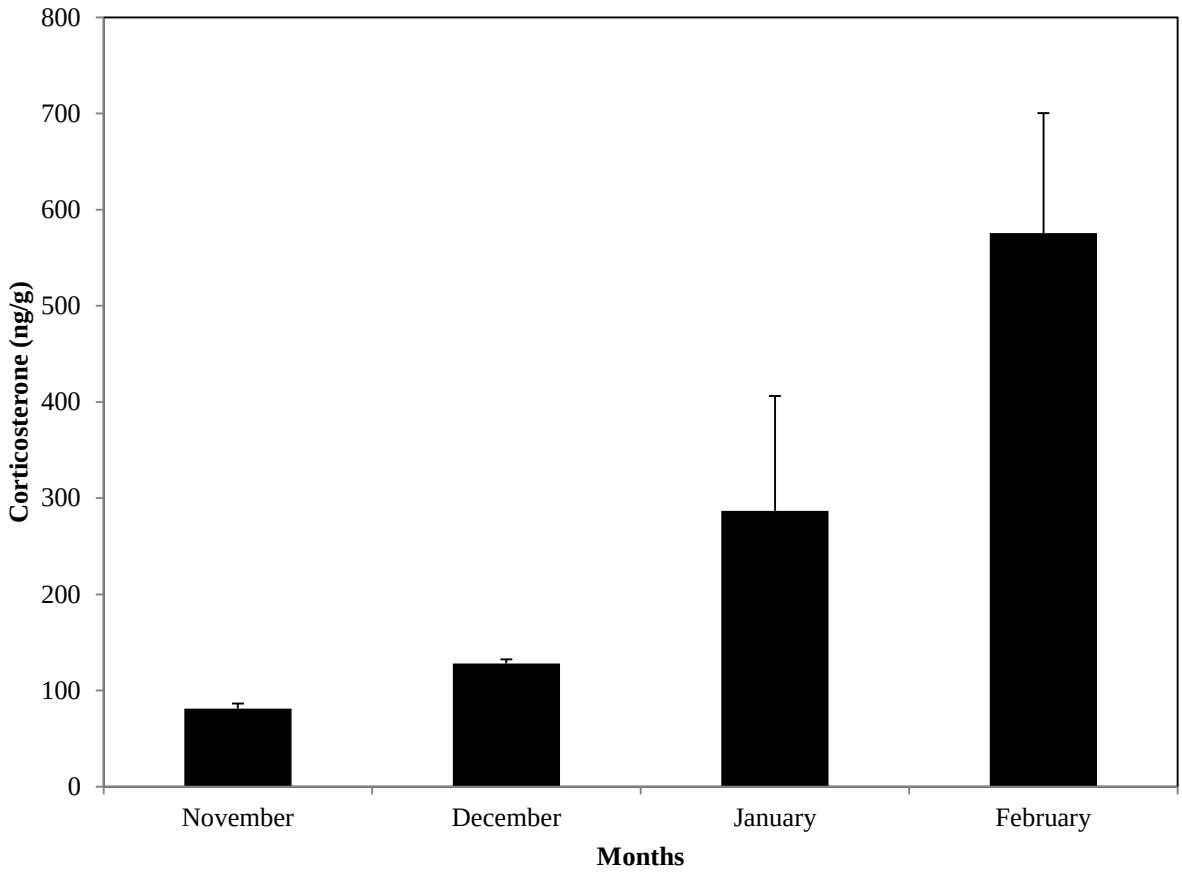


Figure 2.18. Seasonal variation in mean fecal corticosterone concentrations (±SE) expressed in ng of hormone per g of dry feces collected in the six wetlands included in the hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico combining both winters (October to February) of 2007/08 and 2008/09. Outliers were removed beyond 3 SD of the mean.

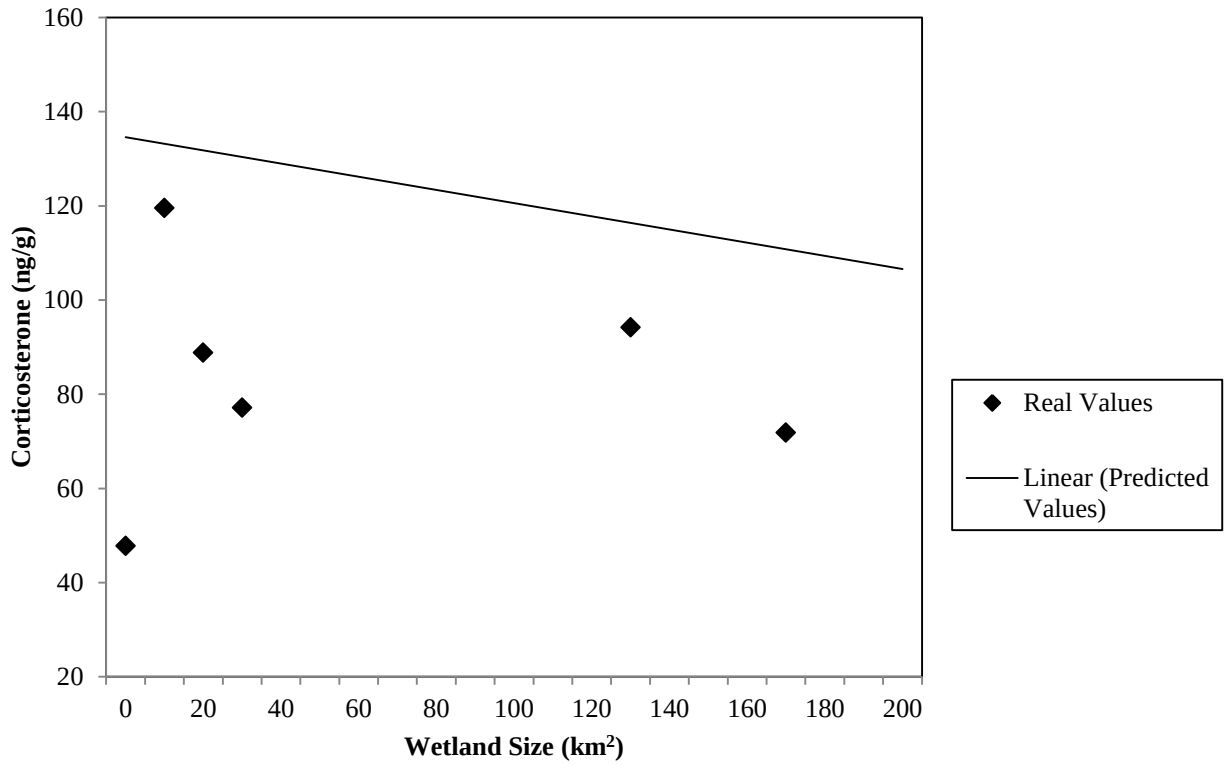


Figure 2.19. Influence of wetland size on mean fecal corticosterone concentrations and best model predicted effects expressed in ng of hormone per g of dry feces collected in the six wetlands included in the hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during winter (October to February) of 2007/08. Outliers were removed beyond 3 SD of the mean.

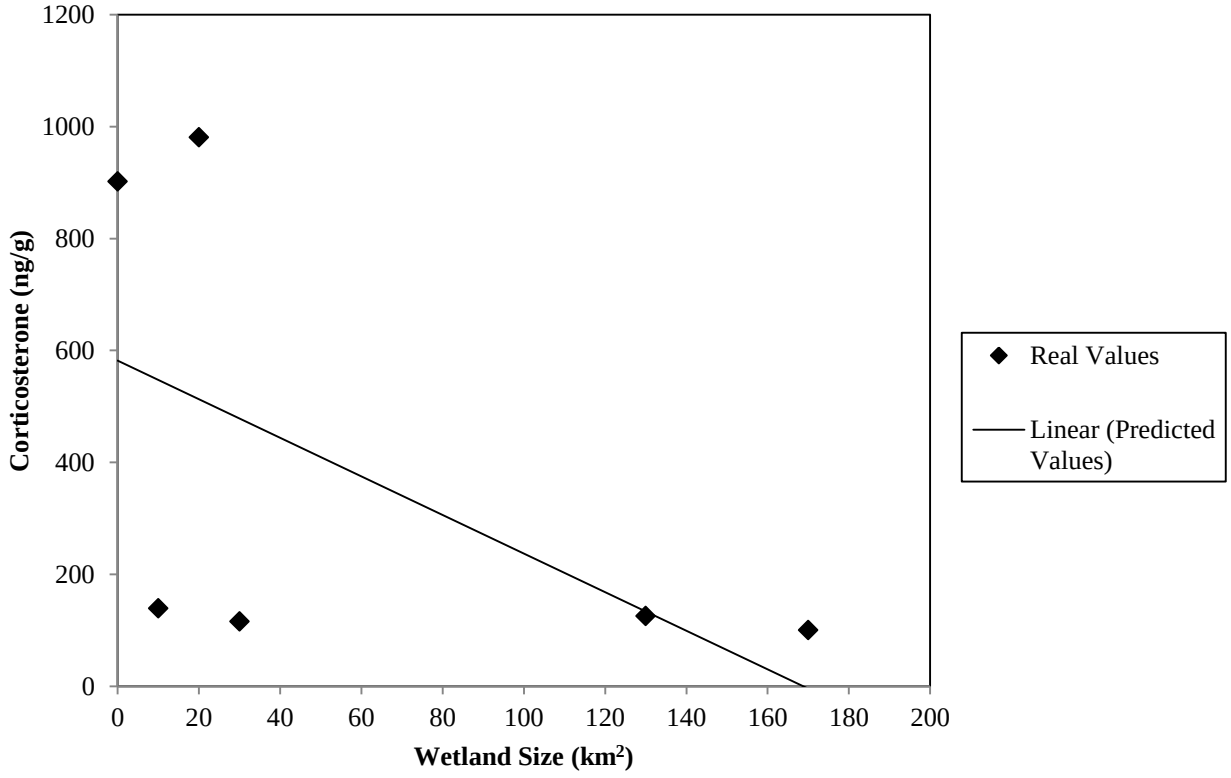


Figure 2.20. Influence of wetland size on mean fecal corticosterone concentrations and best model predicted effects expressed in ng of hormone per g of dry feces collected in the six wetlands included in the hormonal study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during winter (October to February) of 2008/09. Outliers were removed beyond 3 SD of the mean.

# CHAPTER 3: VALIDATION OF THE CORTICOSTERONE ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) TO MEASURE STRESS HORMONES OF SANDHILL CRANES

## ABSTRACT

Fecal glucocorticoid assays have proven to be very valuable as a non-invasive method to assess stress hormones in wild populations because they do not require capturing the animals and has great potential for the field of conservation biology. Steroid immunoassays are becoming increasingly popular as a measure of fecal glucocorticoid metabolites. Among steroid immunoassays, radioimmunoassays (RIA) have a long tradition of performance and are the preferred method among endocrinology laboratories. However, equipment to perform RIA and means to obtain custom-made radio labels are not always available in Third World countries. The aim of this study was to validate an affordable method for measuring glucocorticoids non-invasively in feces of Sandhill Cranes (*Grus canadensis*). I tested a commercially available enzyme-linked immunosorbent assay (ELISA) for corticosterone and compared it with a commercially available radioimmunoassay. I demonstrated a positive correlation between RIA and enzyme immunoassay (EIA; $r = 0.75$, $p < 0.001$) that became stronger when adding an enzyme (i.e., β-Glucuronidase / Arylsulfatase) to hydrolyze the hormone during extraction ($r = 0.90$, $p < 0.001$). However, the correlation between samples that did not contain the enzyme and the samples that contained the enzyme was also strong and positive ($r = 0.90$, $p < 0.001$) when analyzed using EIA, indicating that both groups of samples had similar results. My data suggests that fecal glucocorticoid metabolites can be measured using EIA and that there is no need to add an enzyme during hormone

extraction to obtain reliable results. My results provide valuable information about an affordable technique to perform physiological studies anywhere in the world with minimum laboratory equipment.

## INTRODUCTION

Animals exposed to stressful situations such as food deprivation, weather extremes, predator recognition, capture, and restraint, get their hypothalamic-pituitary-adrenal (HPA) axis activated (Harvey and Hall 1990). This activation releases glucocorticoids that act as physiological mediators to cope with these stressful situations that require modifications of behavior and metabolism. Glucocorticoids, and more specifically corticosterone in birds (Hadley 1996), are the most useful method to assess individuals' responses to stressful situations (Quillfeldt and Mostl 2003).

Fecal glucocorticoid analyses are becoming increasingly popular (Wassser et al. 2000), among the various techniques available to measure the stress response (i.e., adrenocortical activity). One of the advantages of measuring stress levels through fecal samples is it is a non-invasive technique that does not require capturing the animal with its subsequent effect on stress levels due to restraining (Siegel 1995). It is a useful method to study endangered and/or inconspicuous species that can be difficult to capture and for which trapping permits are not feasible. Fecal glucocorticoid studies have been used in Rocky Mountain Bighorn Sheep (*Ovis canadensis canadensis*; Miller et al. 1991), African Wild Dogs (*Lycaon pictus*; Creel et al. 1997), Redpolls (*Acanthis flamea*; Wingfield et al. 1994), Northern Spotted Owl (*Strix occidentalis caurina*; Wasser et al. 1997), and Black Grouse (*Tetrao tetrix*; Baltic et al. 2005).

Fecal glucocorticoid assays have proven to be very valuable as a non-invasive method to assess stress hormones in wild populations, although there are several confounding factors that can affect the results (Millspaugh and Washburn 2004). Confounding factors can be divided into controllable factors such as sampling issues and assay artifacts, and uncontrollable or biological factors. Sex and age are biological confounding factors; both males and females (Romero and Remage-Healey 2000) and adult and juvenile individuals (Dufty and Belthoff 1997) can vary in their adrenocortical response to stress. However, Hartup et al. (2005) did not find a difference in fecal corticoid concentrations across sex or age of captive Whooping Cranes (*Grus americana*). Another confounding factor is the reproductive status of the individual; for example, glucocorticoid baseline circulating levels in lactating females tend to be higher (Kenagy and Place 2000). In contrast, breeding activity in birds and the following parental care require lower levels of corticosterone (Wingfield 2003). Previous studies have also detected that corticosterone levels vary with daily activity rhythms and with the time between excretions (Touma et al. 2003). Seasonal variation in glucocorticoid levels have also been reported in a number of mammalian and avian species such as Carolina Chickadees (*Poecile carolinensis*; Lucas et al. 2006) and tend to be higher during winter independent of local environmental conditions (Romero et al. 1997). Levels of fiber and other nutritional parameters in the diet also influence gut microbial metabolism and therefore glucocorticoid concentrations (Wasser et al. 1993). In addition, nutritional values of food resources for wild animals vary seasonally, making interpretation more difficult. Finally, the excretion route also can have an effect in hormone values because metabolites from circulating hormones may be excreted in different proportions by

different tracts (i.e., urinary and/or gastrointestinal; Wasser et al. 2000). To address the controllable potential sources of variation, I collected the samples following a strict protocol described in the Data Collection section, I assessed corticosterone using duplicates for each sample, and I obtained highly correlated standard curves to improve the predictions. To address the uncontrollable potential sources of variation, I collected enough samples to obtain a representation of the varying physiological status of the population of cranes wintering in the area in general.

Two major techniques are available to measure fecal glucocorticoids, gas chromatography – mass spectrometry, where mixtures of steroid hormone metabolites are analyzed after extraction and derivatization (i.e., transformation of a chemical compound into a product of similar chemical structure; Miksik 1999), and steroid immunoassays where a label and the steroid being measured compete for an antibody binding site (Kellie 1975). Steroid immunoassays are cheaper and allow measurement of many samples in shorter time but they are less specific (Mostl et al. 2005). Furthermore, there are two types of immunoassays, radioimmunoassays (RIA) and enzyme immunoassays (EIA). RIA was the first immunoassay to be developed and therefore has a longer tradition of performance, but it also has higher precision (Mostl et al. 2005). However, a disadvantage of this method is that it generates radioactive waste material for which specific disposal procedures and permits are required. In addition, most studies that use RIA do so using a custom-made corticosterone label which can be very costly (Mostl et al. 2005).

There are two different types of validation for non-invasive hormone measurements such as fecal glucocorticoid analyses, the analytical and the physiological,

or biological, validation. The analytical validation is meant to ensure parallelism, accuracy, and precision of the method being used. The main purpose of this validation is to find out if there are any substances in the extract that could disturb the binding properties of the antibodies (Chard 1995, Goymann 2005). The physiological validation is only required when measuring hormones in fecal samples because the circulating hormone itself is no longer present in excreta but instead metabolites of the original hormone (Goymann et al. 2002a, b, Palme et al. 2005b). The validity of a fecal hormone measurement relies on the assumption that the concentration of hormone metabolites in feces reflects the circulating levels of the actual hormone (Goymann 2005, Mostl et al. 2005). Each hormone typically has several metabolites present in the excreta but their exact identity is not always known. Antibodies for these metabolites are not usually available and instead commercial or custom-made antibodies for the original hormone are used, hoping that the antibodies will cross-react with one or several of the hormone metabolites (Goymann 2005).

The ACTH (adrenocorticotrophic hormone) challenge is the most common method to perform physiological validations in corticosteroid studies. The method involves the administration of high doses of ACTH, a hormone produced and secreted by the pituitary gland to stimulate the cortex of the adrenal gland to produce corticosteroids, to stimulate the circulating levels of corticosterone with the expectation of finding the respective changes in excreted hormone metabolite levels (Goymann 2005). Every species excretes different metabolites that may or may not cross-react with the antibody used in the assay, hence the importance of the validation of antibodies for each new species studied (Buchanan and Goldsmith 2004, Palme 2005, Touma and Palme 2005).

Ludders et al. (1998) performed a physiological validation and demonstrated that fecal corticosterone measures provide a reliable measure of the glucocorticoid stress response in Florida Sandhill Cranes (*Grus canandensis pratensis*).

The goal of my study was to validate a method for measuring glucocorticoids non-invasively in feces of Sandhill Cranes (*Grus canadensis*). The specific objectives were to (1) validate the use of an affordable and commercially available corticosterone kit to measure stress hormones in Sandhill Cranes, (2) test whether levels of corticosterone metabolites measured with EIA assays were comparable to levels measured with RIA, and (3) test the effects of adding an enzyme to fecal samples to achieve enzyme hydrolysis during hormonal extraction.

## MATERIALS AND METHODS

### *Study Area*

I sampled stress hormones of Sandhill Cranes in central Nebraska where the Mid-continent Population stages during the spring migration for 4-6 weeks every year (Krapu et al. 1984). I collected my samples from five sites along the Platte River. The sites included a roost in front of The Crane Trust (40°46’49.12’’N; 98°28’21.69’’W), a roost in front of the Audubon Rowe Sanctuary (40°40’27.74’’N; 98°52’52.77’’W), a loafing area at The Crane Trust (40°47’43.23’’N; 98°27’36.71’’W), a loafing area at Mormon Island State Recreation Area (40°48’2.43’’N; 98°25’5.80’’W), and a corn field in The Crane Trust property (Uridil; 40°43’40.19’’N; 98°37’53.17’’W). In addition, I also analyzed samples from Sandhill Cranes collected from the Wisconsin River near Briggsville, Wisconsin (43°38’27.79’’N; 89°32’48.71’’W).

*Data Collection*

Wisconsin samples.—Personnel of the Field Ecology Department of the International Crane Foundation (ICF) collected fecal samples of Sandhill Cranes from roosts sites in the Wisconsin River between June and November 2008. They collected fresh fecal samples less than 2 h old during the morning hours and placed them in sterile whirl-pak bags. They froze the samples at -20°C within 2 h after collection and kept them frozen until analysis.

Nebraska samples.—I collected samples of feces of Sandhill Cranes from five sites along the Platte River in Nebraska during March and April in 2009. I located the sites by direct observation of flocks of cranes taking off at dawn for the roost sites and at dusk for the loafing sites. The condition of fecal samples that lay on the ground for a long period of time and are exposed to high temperatures can be compromised due to biochemical changes in immunoreactivity and degradation of steroids (Matkovics 1972, Terio et al. 2002). Therefore, I waited for cranes to leave a site and then collected fresh samples that had been deposited during the morning and the evening hours to avoid microbes in the feces to start metabolizing the fecal glucocorticoids (Woods 1975, Mostl et al. 1999, Washburn and Millspaugh 2002). In addition, previous studies have detected that corticosterone levels vary with daily activity rhythms and with the time between excretions (Touma et al. 2003). The metabolic rate of songbirds drops more than normal during the night (i.e., when birds cannot forage) contributing to energy saving needed for the next morning (Astheimer et al. 1992). I collected the samples during different times of the day to include a range of stress levels that could be detected and compared using both measuring methods. Another issue concerning sample condition is the exposure to

precipitation due to added moisture providing a suitable growth environment for microbes and detritivores (Washburn and Millspaugh 2002). I avoided collecting samples during rainy or snowy days when samples could get wet before collection. Cranes roost in the river and deposit most of their feces into water. To ensure good quality of samples, I only collected fresh feces (i.e., less than two hours after the cranes left their roost) that were deposited in sand banks in the Platte River.

I collected the samples using a 3 oz sterile stainless steel scoop (AMS, American Falls, Idaho) and placed the samples in individual and sterile 4 oz plastic whirl-pak bags (Nasco Whirl-Pak, Fort Atkinson, Wisconsin); numbered, dated, and assigned a location name for each one. I dipped and shook the scoop into a container filled with ethyl alcohol 90% after each collecting in order to clean the scoop and avoid contamination between samples. I refilled the container with new alcohol between sites. Samples need to be frozen as quick as possible to preserve their fecal glucocorticoid metabolite (Terio et al. 2002, Millspaugh and Washburn 2004); therefore I used a 12 V AC/DC portable freezer (Engel Freezers, Jupiter, Florida) to keep the samples frozen while transportation between the field and the laboratory. I followed protocols of Millspaugh and Washburn (2004), and I froze my samples without adding any chemical treatment (e.g., acetic acid or ethanol: Khan et al. 2002, Lynch et al. 2003). I was then able to extract the fecal glucocorticoid metabolite at a later date, and the metabolite remained stable until I performed the analyses (Lynch et al. 2003). I stored the samples at –20 °C in the laboratory of The Crane Trust for better preservation and to avoid fungal development until processing (Khan et al. 2002)

*Data Analysis*

Hormone extraction.—I analyzed the samples at the Endocrinology Lab of the Endangered Species Research Center and Veterinary Hospital of the Saint Louis Zoo, Saint Louis, Missouri. In the laboratory, I thawed the fecal samples and removed any debris with a pair of thin tweezers. Avian fecal samples contain a white body of uric acid adhered to the excreta, which I assumed to be constant in proportion to the excreta volume across samples. Hormone metabolites are excreted in different amounts in urine and feces (Wasser et al. 2000). I prepared the excreta as a whole, following the protocols of Ludders et al. (2001) and Washburn et al. (2003) who suggested the technique provides a more complete estimate of total glucocorticoid metabolites (see Millspaugh and Washburn 2004, for critique). Therefore, I mixed the samples thoroughly inside the whirl-pak bags to evenly distribute urates and fecal material and ensure homogeneity.

The first step in the quantification of steroid hormones is the extraction of the hormone (e.g., corticosterone) from the biological medium where it is confined (e.g., plasma, urine, or excreta). I extracted the stress hormone from each fecal sample by placing 0.5 g of sample into a sterile 20 ml disposable scintillation vial (Wheaton, Millville, New Jersey) using a spatula and cleaning it between samples with wipes (Kimberly-Clark Kimwipes, Neenah, Wisconsin). The empty vials had been previously weighed on an analytical precision balance and numbered. I then divided the samples into two groups that received different treatments. The first group did not receive an enzyme (hereafter Non-enzyme Samples) although the second group received an enzyme (hereafter Enzyme Samples). I added 25 µl of β-Glucuronidase / Arylsulfatase (Roche Diagnostics, Indianapolis, Indiana) to Enzyme Samples to test the effects of enzyme

hydrolysis in fecal extractions. As mentioned earlier, bird droppings contain feces mixed with urates. Hormones contained in the urine are mostly found in conjugated form (e.g., pregnanediol glucuronide instead of progesterone; estrone sulfate instead of estradiol; Silverthorn 2010). Adding an enzyme to the sample hydrolyzes the conjugates and may help extract more of the original hormone to be measured. In addition, I added 5 ml of fecal steroid extraction buffer (Phosphate / Methanol) without Sodium azide to Non-enzyme Samples and 2.5 ml of fecal steroid extraction buffer with 1 g Sodium azide to Enzyme Samples. Sodium azide ($NaN_3$) inhibits bacterial growth but it appears to interact with the enzyme of an ELISA kit (J. Bauman, pers. comm.). The fecal steroid extraction buffer consisted of 1 l $H_2O$, 8.75 g NaCl, 5.75 g $NaH_2PO_4*H_2O$, 8.61 g $Na_2HPO_4$, 0.5 ml Tween-20 detergent solution (i.e., polyoxyethylene 20 sorbitan monolaurate), 1 g BSA (Bovine Serum Albumin; Sigma-Aldrich, Saint Louis, Missouri) to prevent adhesion of the enzyme to tubes and pipette surfaces, and 50% methanol (i.e., methyl alcohol; protocol modified from Shideler et al. 1994).

I placed all the samples in a vortex mixer for 1 min until the samples had become well dispersed. I then shook Non-enzyme Samples in an orbital shaker (New Brunswick Scientific, Edison, New Jersey) at 200 rpm overnight (i.e., about 16 h). Meanwhile, I placed Enzyme Samples in the oven at 37°C to incubate overnight without shaking them.

The next day, I let Non-enzyme Samples rest for 1 h before I transferred the supernatant into 12 by 75 polypropylene culture tubes. I dried the remaining scintillation vials in a vented oven at 100 °C overnight. Meanwhile, I centrifuged the samples at 4,000 rpm and 4°C for 1 h to further separate the supernatant from the rest of the sample. After centrifugation, I transferred the supernatant once again into 3.6 ml cryovials with

caps (Wheaton, Millville, New Jersey). I then stored the vials in the freezer at -20 °C to stabilize the samples until I could perform the assays (protocol modified from Bauman and Hardin 1998b).

I added 2.5 ml methanol to Enzyme Samples that had been incubated overnight. I shook them in the orbital shaker at 200 rpm for 4 h. I let the samples rest for 1 h before I transferred the supernatant into 12 by 75 polypropylene culture tubes. I dried the remaining scintillation vials in a vented oven at 100 °C overnight. Meanwhile, I centrifuged the samples at 4,000 rpm and 4°C for 1 h to further separate the supernatant from the rest of the sample. After centrifugation, I transferred the supernatant once again into 3.6 ml cryovials with caps. I then stored the vials in the freezer at -20 °C to stabilize the samples until I could perform the assays (Bauman and Hardin 1998b).

The following day, I removed the scintillation vials from the oven and allowed them to achieve room temperature. I then weighed them recording both the weight of the vial and of the dried feces. I subtracted the weight of the empty vials recorded earlier to determine the weight of the dry feces. This value was later used in the calculations of hormone levels.

Once I extracted the hormone from the fecal samples I performed two types of assays on each extraction; an enzyme immunoassay (EIA) and a radioimmunoassay (RIA; Mostl et al. 2005). Both kits are used for quantitative analysis of corticosterone levels in biological fluids and operate on the basis of competition between the label or enzyme conjugate and the steroid being measured (e.g., corticosterone) for a limited number of antibody binding sites on a coated plate (Kellie 1975).

EIA assay.—For the EIA, I used a non-species specific commercially available corticosterone Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (Corticosterone Kit, Neogen Corporation, Lexington, Kentucky). The kit uses corticosterone horseradish peroxidase concentrate as the label for corticosterone. This label has a cross reactivity of 100% with the excreted corticosterone metabolites. Corticosterone hormone itself is not present in bird feces, instead corticosterone metabolites are excreted (Palme et al. 2005a). The assay relies on the cross-reaction with the metabolites and so the higher the percentage of cross-reaction the better the kit will measure the glucocorticoid metabolite level.

I added 40 samples and eight standards of known corticosterone concentration in duplicates to a 96-well corticosterone antibody coated microplate. I added enzyme conjugate to each well using an 8-multichannel pipette with a volume range from 30 to 300 µl (Eppendorf, Hamburg, Germany) to ensure the same time of reaction between wells and mixed it shaking gently with the use of a microplate shaker. I then let the microplate incubate at room temperature for 1 hour. The competition for the binding sites takes place during the incubation period. After incubation I washed the microplate three times with a wash buffer to remove all the unbound material. I added a substrate (i.e., tetramethylbenzidine and hydrogen peroxide) with a multichannel pipette to react and develop color at room temperature for 30 min to detect the bound material left in the wells, and then I added 1N HCl to stop the enzyme reaction. I shook the microplate again to ensure uniform color throughout each well and read the plate using a microplate reader with a 650-nm filter. I used a microplate reader to measure the amount of light

that is absorbed by the samples (i.e., optical density) and compare it with the amount absorbed by the standards.

For the calculations, I averaged the optical density readings of the duplicates of the standards and calculated the percent of maximal binding (%$B/B_0$) by dividing the averages of each standard ($B_1$ to $B_7$) by the average of the standard with zero concentration of corticosterone ($B_0$) multiplied by 100. I calculated a standard curve between the percent of maximal binding and the concentration of corticosterone (ng/ml) of each standard and obtained a linear regression equation to calculate the concentration of corticosterone for each sample (Appendix D and E). I directly diluted all the samples with a dilution factor of four to make sure the concentration of corticosterone would fall inside the standard curve. I then adjusted the new concentration obtained for that sample multiplying it by four. However, if the concentration of a sample still felt outside the standard curve I diluted that sample with a greater dilution factor and repeated the assay.

RIA assay.—For the RIA, I used a non-species specific commercially available corticosterone RIA kit (Corticosterone Double Antibody I-125 RIA Kit, MP Biomedicals (former ICN), Santa Ana, California). This kit uses Iodine-125 (I-125) as the radioisotope label for corticosterone. This label has been shown to have a cross reactivity of 100% with excreted corticosterone metabolites in a wide variety of species (Wasser et al. 2000). I followed the manufacturer's assay and the Saint Louis Zoo protocols (Bauman and Hardin 1998a).

Statistical analyses.—I calculated a regression equation between the EIA and RIA assay methods using R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria; R Development Core Team 2011). I estimated the Pearson correlation

coefficient between EIA and RIA as a measure of the linear dependence between the two methods. I also calculated a regression equation and a linear correlation coefficient between the Non-enzyme and Enzyme Samples treatment using both assay methods. Finally, I calculated a correlation coefficient between Wisconsin samples analyzed using EIA and RIA assays to provide a different validation using samples from a different location and from a different population. I executed the analysis on log transformed corticosterone levels to retain normality.

**RESULTS**

I collected 100 fresh fecal samples (IB-1 to IB-100) in all sites during the spring migration of 2009. In the lab, I ran a total 4 ELISA's for 160 fecal samples after repeating samples that needed extra dilution. I ran the first 100 samples as Non-enzyme Samples and I repeated the first 50 samples (IB-301 to IB-350) as Enzyme Samples. For Non-enzyme Samples, the mean fecal glucocorticoid concentration was 49.2±4.1 ng/g dry feces ($n$ = 100, median = 41.5) and 6.5±0.4 ng/g dry feces ($n$ = 100, median = 5.4) using EIA and RIA assays respectively. I found a positive significant correlation between the two methods ($r$ = 0.75, $p < 0.001$; Fig. 1).

Regarding Enzyme Samples, the mean fecal glucocorticoid concentration was 37.7±3.9 ng/g dry feces ($n$ = 50, median = 35.5) and 12.2±1.3 ng/g dry feces ($n$ =50, median = 11.8) using EIA and RIA assays respectively. This correlation was stronger than the first one ($r$ = 0.90, $p < 0.001$; Fig. 2).

The addition of enzyme had a greater impact on RIA analyzed samples than on EIA samples, when comparing between Non-enzyme and Enzyme Samples. Non-enzyme Samples analyzed on EIA registered a mean fecal glucocorticoid concentration

of 56.7±6.5 ng/g dry feces ($n$ = 50, median = 52.8) and Enzyme Samples registered 37.7±3.9 ng/g dry feces ($n$ = 50, median = 35.5). The correlation between Non-enzyme and Enzyme Samples with EIA was 0.90 and significant ($p < 0.001$; Fig. 3). Non-enzyme Samples analyzed on RIA registered a mean fecal glucocorticoid concentration of 7.7±0.7 ng/g dry feces ($n$ = 50, median = 8.0) and Enzyme Samples registered 12.2±1.3 ng/g dry feces ($n$ = 50, median = 11.8). The correlation between Non-enzyme and Enzyme Samples with RIA was 0.86 and also significant ($p < 0.001$; Fig. 4).

I run 10 samples from Wisconsin using EIA (81.5±17.7 ng/g dry feces, $n$ = 10) and RIA (23.4±8.0 ng/g dry feces, $n$ = 10). I found a positive significant correlation of 0.97 ($p<0.001$; Fig. 5) between the two methods.

**DISCUSSION**

Objective (1).—This is the first study to validate the use of a commercially available corticosterone kit as a method for measuring glucocorticoids non-invasively in feces of Sandhill Cranes. Most steroid studies use a custom-made antibody that it is either species-specific or has been validated for that species (Goymann et al. 1999, Goymann et al. 2002a, Quillfeldt and Mostl 2003). The results of this validation demonstrate that stress levels in wild birds can be measured using an EIA assay with a commercially available corticosterone kit. When using a corticosterone kit, I recommend following the instructions of the manufacturer. The only modification to the kit that I recommend is to make your own extraction buffer since manufacturers do not usually disclose the composition of the buffer provided with their kit. A corticosterone extraction buffer should contain PBS (i.e., phosphate buffered saline) powder and methanol or any other solvent.

Objective (2).—This is also the first study to compare fecal glucocorticoid metabolites analyses using two different types of immunoassay, EIA and RIA. The first and most commonly used assay to measure fecal glucocorticoid metabolites has traditionally been RIA (Wingfield et al. 1994, Creel et al. 1997, Wasser et al. 1997, Washburn et al. 2003); however, the benefits provided by EIA are increasing its popularity among biochemistry assays. EIA offers advantages in terms of health safety, since it does not generate radioactive waste material, and financial cost while still providing accurate measures of steroid metabolite concentrations (Goymann 2005). I showed that the results obtained using both methods are comparable despite differences in the values returned by each assay. The results of my study indicated that fecal glucocorticoid concentration values are higher when using EIA than RIA. However, the correlation between the two methods is significant enough to accept both methods and consider results obtained with EIA as accurate as the traditional RIA. Comparison between studies that have used a different assay should only be done in respect to relative comparisons. For the purpose of many conservation studies such comparison may be sufficient, but in general, comparison between results obtained through different methods is not advisable due to the large difference in absolute values.

Objective (3).—The use of an enzyme had opposite effects on samples analyzed using EIA or RIA. For EIA samples, corticosterone estimates decreased and for RIA samples, corticosterone estimates increased with the use of an enzyme. Although the addition of an enzyme brought the two methods closer to each other resulting in a stronger correlation than without an enzyme, I do not recommend adding a custom-made enzyme to the samples when using EIA for future validation studies. The purpose of

adding an enzyme is usually to hydrolyze the conjugates in the sample and aid in the extraction of hormone metabolites from the feces. It is possible that in the case of EIA, enzyme conjugate (i.e., horseradish peroxidase) provided with the kit is already added during the hormone extraction separating most of the corticosterone to be measured from the samples. The addition of an extra custom-made enzyme (i.e., β-Glucuronidase / Arylsulfatase) may interfere with the separation of the metabolites and mask the optical density measured by the microplate reader returning a lower value. On the other hand, the custom-made enzyme was the only enzyme catalyzing the hormone extraction process in RIA; therefore, it was expected that the values of corticosterone metabolite concentration would be higher.

The results of this validation provide information for physiological studies performed in Third World countries where RIA assay instruments may not be available. The preferred methodology should still be RIA because of the level of precision that the assay provides (Mostl et al. 2005), but in those instances when such technology is not available, the alternative assay can be EIA. Measurement of stress hormones in the wild can be performed using EIA once the target species has been tested with an ACTH challenge for a physiological validation. Such validations can be performed in individuals kept in captivity. Fecal glucocorticoid metabolite assays have been validated for several species, including the Northern Spotted Owl (Wasser et al. 1997), Florida Sandhill Crane (Ludders et al. 1998), Greylag Geese (*Anser anser*; Frigerio et al. 2001), and Mourning Dove (*Zenaida macroura*; Washburn et al. 2003), providing the first step in fecal glucocorticoid analyses.

Regardless of the method used, it must be noted that because each assay utilizes different antibodies with varying affinity for glucocorticoid metabolites, it is not appropriate to compare absolute values from two different studies if two different assays have been used (Millspaugh and Washburn 2004, Goymann 2005).

**LITERATURE CITED**

Astheimer, L. B., W. A. Buttemer, and J. C. Wingfield. 1992. Interactions of corticosterone with feeding, activity and metabolism in passerine birds. Ornis Scandinavica 23:355-365.

Baltic, M., S. Jenni-Eiermann, R. Arlettaz, and R. Palme. 2005. A noninvasive technique to evaluate human-generated stress in the black grouse. Annals of the New York Academy of Sciences 1046:81-95.

Bauman, J. E., and A. Hardin. 1998a. Measurement of steroids in animal feces with commercially available RIA kits intended for use in human serum. Journal of Clinical Lingand Assay 21:83.

Bauman, J. E., and A. Hardin. 1998b. Measurement of steroids in animal feces with commercially available RIA kits intended for use in human serum. Journal of Clinical Ligand Assay 21:83.

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

Chard, T. 1995. An introduction to radioimmunoassay and related techniques. Elsevier, Amsterdam, The Netherlands.

Creel, S., N. M. Creel, and S. Monfort. 1997. Radiocollaring and stress hormones in African wild dogs. Conservation Biology 11:544-548.

Dufty , J. A. M., and J. R. Belthoff. 1997. Corticosterone and the stress response in young western screech-owls: effects of captivity, gender, and activity period. Physiological Zoology 70:143-149.

Frigerio, D., E. Mostl, and K. Kotrschal. 2001. Excreted metabolites of gonadal steroid hormones and corticosterone in Greylag Geese (*Anser anser*) from hatching to fledging. General and Comparative Endocrinology 124:246-255.

Goymann, W. 2005. Noninvasive monitoring of hormones in bird droppings: Physiological validation, sampling, extraction, sex differences, and the influence of diet on hormone metabolite levels. Annals of the New York Academy of Sciences 1046:35-53.

Goymann, W., E. Mostl, and E. Gwinner. 2002a. Corticosterone metabolites can be measured noninvasively in excreta of European stonechats (*Saxicola torquata rubicola*). Auk 119:1167-1173.

Goymann, W., E. Mostl, and E. Gwinner. 2002b. Non-invasive methods to measure androgen metabolites in excrements of European stonechats, *Saxicola torquata rubicola*. General and Comparative Endocrinology 129:80-87.

Goymann, W., E. Mostl, T. Van't Hof, M. L. East, and H. Hofer. 1999. Noninvasive fecal monitoring of glucocorticoids in spotted hyenas, *Crocuta crocuta*. General and Comparative Endocrinology 114:340-348.

Hadley, M. E. 1996. Endocrinology. Prentice-Hall, Upper Saddle River, New Jersey, USA.

Hartup, B. K., G. H. Olsen, and N. M. Czekala. 2005. Fecal corticoid monitoring in whooping cranes (*Grus americana*) undergoing reintroduction. Zoo Biology 24:15-28.

Harvey, S. J., and T. R. Hall. 1990. Hormones and stress in birds: activation of the hypothalamopituitary-adrenal axis. Pages 453-460 *in* Progress in comparative endocrinology: proceedings of the eleventh international symposium on comparative endocrinology. Willy-Liss, Inc., New York.

Kellie, A. E. 1975. Methods of steroid analysis. II. Competitive binding. Pages 211-226 *in* H. L. J. Makin, editor. Biochemistry of Steroid Hormones. Blackwell Scientific Publishing, Oxford.

Kenagy, G. J., and N. J. Place. 2000. Seasonal changes in plasma glucocorticosteroids of free-living yellow pine chipmunks: effects of reproduction and capture and handling. General and Comparative Endocrinology 117:189-199.

Khan, M. Z., J. Altmann, S. S. Isani, and J. Yu. 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. General and Comparative Endocrinology 128:57-64.

Krapu, G. L., D. E. Facey, E. K. Fritzell, and D. H. Johnson. 1984. Habitat use by migrant sandhill cranes in Nebraska. Journal of Wildlife Management 48:407-417.

Lucas, J. R., T. M. Freeberg, J. Egbert, and H. Schwabl. 2006. Fecal corticosterone, body mass, and caching rates of Carolina chickadees (*Poecile carolinensis*) from disturbed and undisturbed sites. Hormones and Behavior 49:634-643.

Ludders, J. W., J. A. Langenberg, N. M. Czekala, and H. N. Erb. 2001. Fecal corticosterone reflects serum corticosterone in Florida sandhill cranes. Journal of Wildlife Diseases 37:646-652.

Ludders, J. W., J. A. Langenberg, N. M. Czekala, H. N. Erb, and H. McCormick. 1998. Serum corticosterone response to adrenocorticotropic hormone stimulation in Florida sandhill cranes. Journal of Wildlife Diseases 34:715-721.

Lynch, J. W., M. Z. Khan, J. Altmann, M. N. Njahira, and N. Rubenstein. 2003. Concentrations of four fecal steroids in wild baboons: short-term storage conditions and consequences for data interpretation. General and Comparative Endocrinology 132:264-271.

Matkovics, B. 1972. In vitro transformation of steroids as a substitute of microbial transformation. Steroids Lipids Research 3:1-7.

Miksik, I. 1999. Separation and identification of corticosterone metabolites by liquid chromatography-electrospray ionization mass spectrometry. Journal of Chromatography B: Biomedical Sciences and Applications 726:59-69.

Miller, M. W., N. T. Hobbs, and M. C. Sousa. 1991. Detecting stress responses in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*): reliability of cortisol concentrations in urine and feces. Canadian Journal of Zoology 69:15-24.

Millspaugh, J. J., and B. E. Washburn. 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. General and Comparative Endocrinology 138:189-199.

Mostl, E., S. Messmann, E. Bagu, C. Robia, and R. Palme. 1999. Measurement of glucocorticoid metabolite concentrations in faeces of domestic livestock. Journal of Veterinary Medicine A 46:621-631.

Mostl, E., S. Rettenbacher, and R. Palme. 2005. Measurement of corticosterone metabolites in birds' droppings: An analytical approach. Annals of the New York Academy of Sciences 1046:17-34.

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

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr, and E. Mostl. 2005a. Stree hormone in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 1040:162-171.

Palme, R., S. Rettenbacher, C. Touma, S. M. El-Bahr, and E. Mostl. 2005b. Stress hormones in mammals and birds: Comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Annals of the New York Academy of Sciences 1040:162-171.

Quillfeldt, P., and E. Mostl. 2003. Resource allocation in Wilson's storm-petrels *Oceanites oceanicus* detemined by measurement of glucocorticoid excretion. Acta Ethologica 5:115-122.

R Development Core Team. 2011. R : A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Romero, L. M., M. Ramenofsky, and J. C. Wingfield. 1997. Season and migration alters the corticosterone response to capture and handling in an Arctic migrant, the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). comparative Biochemistry and Physiology C 116:171-177.

Romero, L. M., and L. Remage-Healey. 2000. Daily and seasonal variation in response to stress in captive starlings (*Sturnus vulgaris*): corticosterone. General and Comparative Endocrinology 119:52-59.

Shideler, S. E., A. Savage, A. M. Ortuño, E. A. Moorman, and B. L. Lasley. 1994. Monitoring female reproductive function by measurement of fecal estrogen and progesterone metabolites in the white-faced saki (*Pithecia pithecia*). Amerian Journal of Primatology 32:95-108.

Siegel, H. S. 1995. Stress, strains and resistance. British Poultry Science 36:3-22.

Silverthorn, D. U. 2010. Human Physiology: an integrated approach. 5th edition. Pearson Education, Inc., San Francisco, California.

Terio, K. A., J. L. Brown, R. Moreland, and L. Munson. 2002. Comparison of different drying and storage methods on quantifiable concentrations of fecal steroids in the cheetah. Zoo Biology 21:215-222.

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

Touma, C., N. Sachser, E. Mostl, and R. Palme. 2003. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. General and Comparative Endocrinology 130:267-278.

Washburn, B. E., and J. J. Millspaugh. 2002. Effects of simulated environmental conditions on glucocorticoid metabolite measurements in white-tailed deer feces. General and Comparative Endocrinology 127:217-222.

Washburn, B. E., J. J. Millspaugh, J. H. Schulz, S. B. Jones, and T. Mong. 2003. Using fecal glucocorticoids for stress assessment in mourning doves. The Condor 105:696-706.

Wasser, S. K., K. Bevis, G. King, and E. Hanson. 1997. Noninvasive physiological measures of disturbance in the Northern Spotted Owl (*Strix occidentalis caurina*). Conservation Biology 11:1019-1022.

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

Wasser, S. K., R. Thomas, P. P. Lair, C. Guidry, J. Southers, J. Lucas, D. E. Wildt, and S. L. Monfort. 1993. Effects of dietary fibre on faecal steroid measurements in baboons (*Papio cynocephalus cynocephalus*). Journal of Reproduction and Fertility 97:569-574.

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

Wingfield, J. C. 2003. Control of behavioural strategies for capricious environments. Animal Behaviour 66:807-816.

Wingfield, J. C., P. Deviche, S. Sharbaugh, L. B. Astheimer, R. Holberton, R. Suydam, and K. Hunt. 1994. Seasonal changes of the adrenocortical responses to stress in redpolls, *Acanthis flamea*, in Alaska. Journal of Experimental Zoology 270:372-380.

Woods, G. F. 1975. Chemical and microbiological transformation of steroids. Pages 5-10 *in* E. H. D. Cameron, S. G. Hillier, and K. Griffiths, editors. Steroid Immunoassay. Alpha Omega, Cardiff, UK.

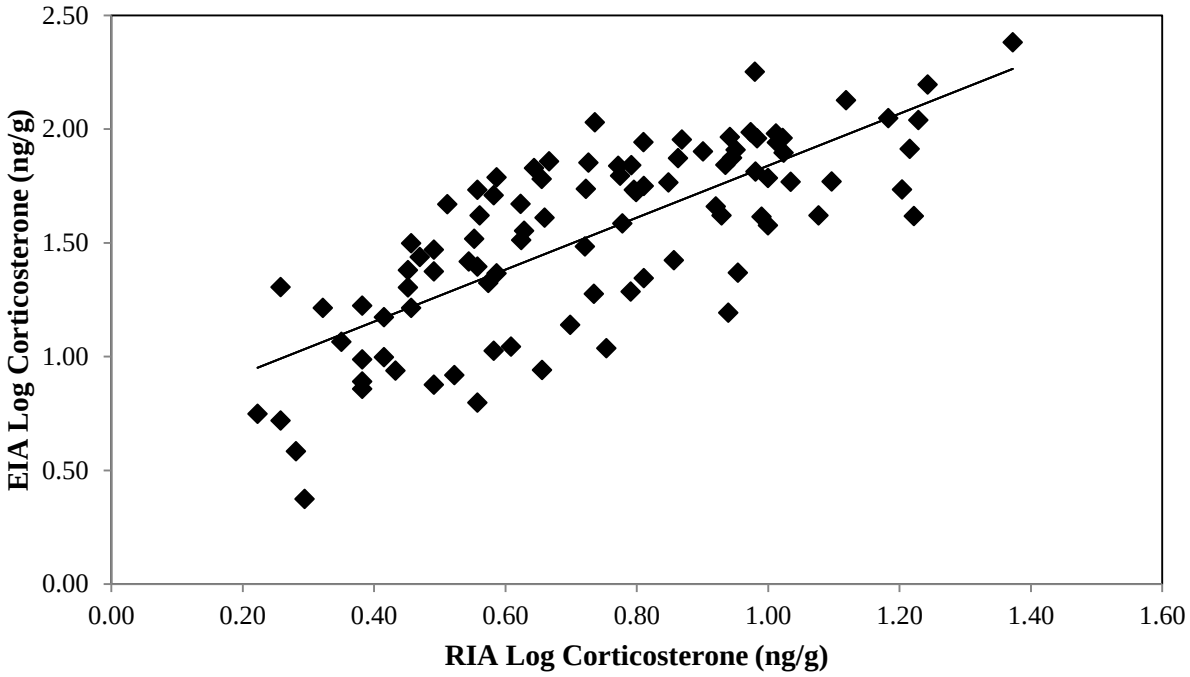


Figure 3.1. Relationship between fecal corticosterone concentrations analyzed with enzyme immunoassay (EIA) and radioimmunoassay (RIA). Corticosterone is expressed in ng of hormone per g of dry feces of Sandhill Cranes (*Grus canadensis*) collected in Nebraska during March and April of 2009. Pearson's correlation coefficient = 0.75; y = 1.1425x + 0.6969. Data were log transformed to retain normality. Samples did not contain β-Glucuronidase / Arylsulfatase enzyme.

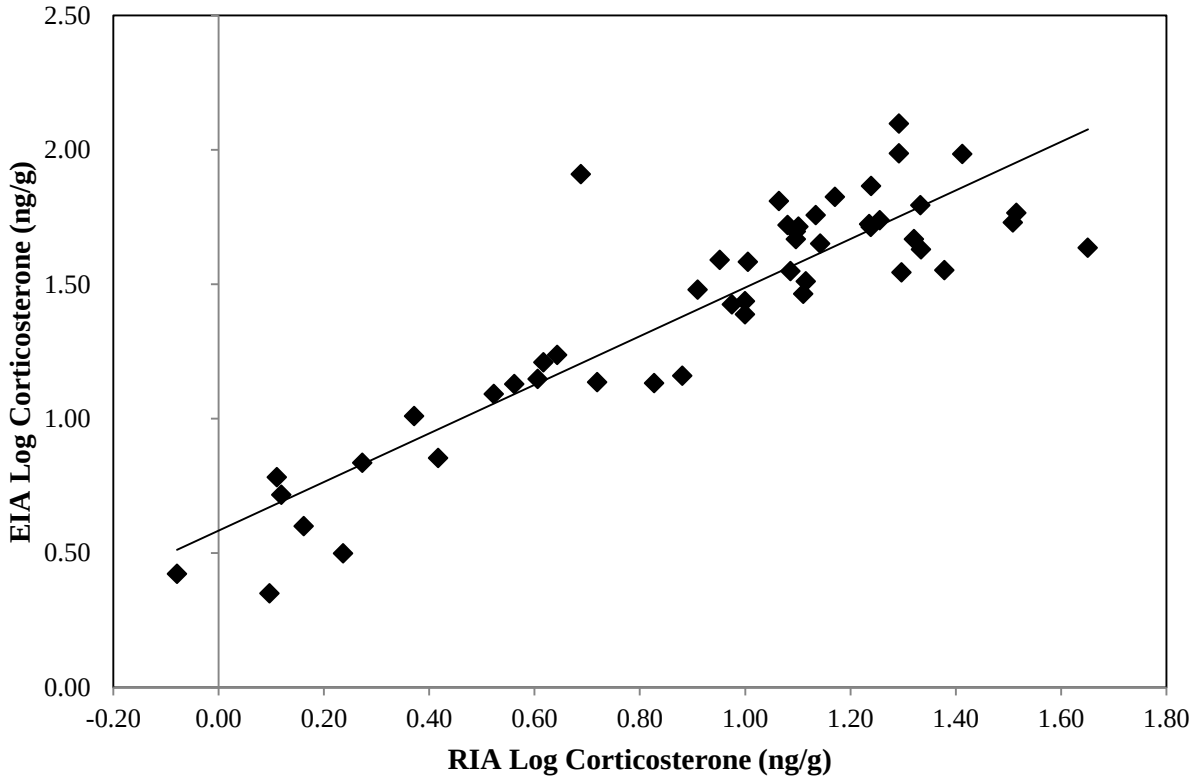


Figure 3.2. Relationship between fecal corticosterone concentrations analyzed with enzyme immunoassay (EIA) and radioimmunoassay (RIA). Corticosterone is expressed in ng of hormone per g of dry feces of Sandhill Cranes (*Grus canadensis*) collected in Nebraska during March and April of 2009. Pearson's correlation coefficient = 0.90; y = 0.9038x + 0.584. Data were log transformed to retain normality. Samples contained β-Glucuronidase / Arylsulfatase enzyme.

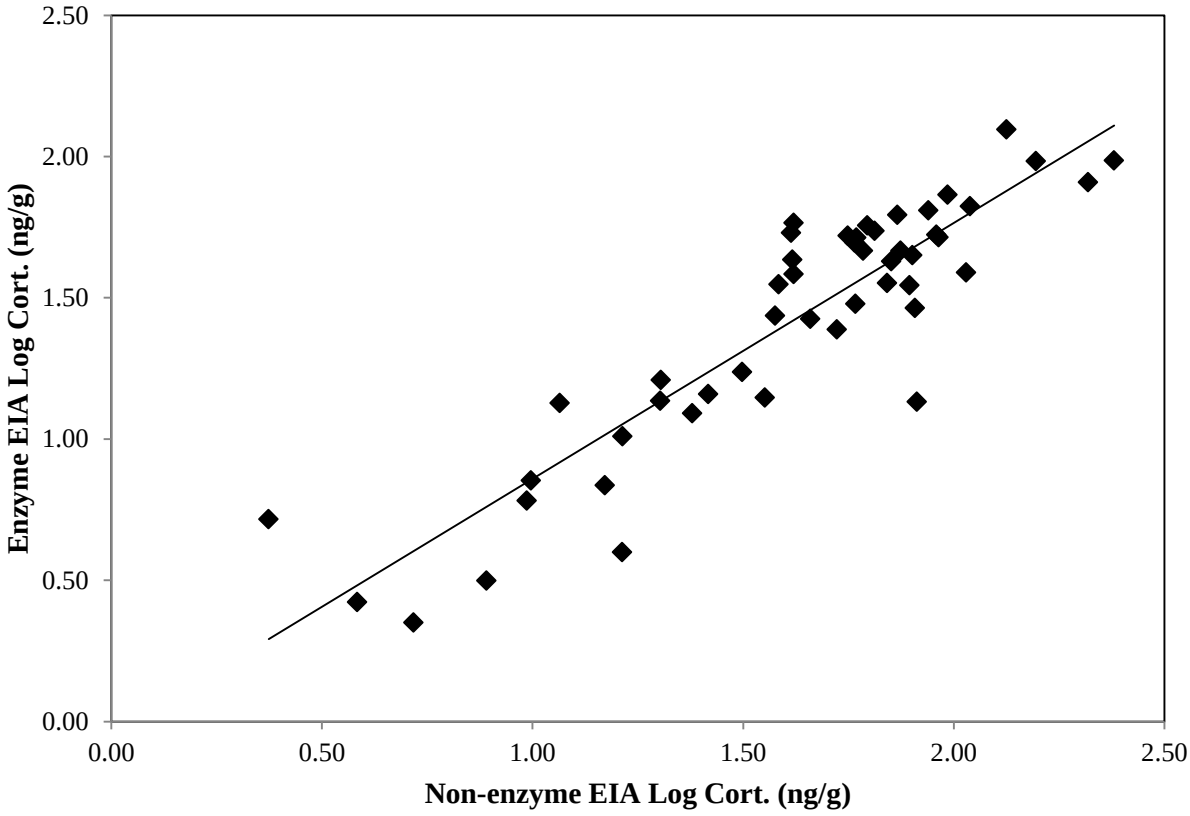


Figure 3.3. Relationship between fecal corticosterone concentrations with and without enzyme analyzed with enzyme immunoassay (EIA). Corticosterone is expressed in ng of hormone per g of dry feces of Sandhill Cranes (*Grus canadensis*) collected in Nebraska during March and April of 2009. Pearson's correlation coefficient = 0.90; y = 0.9055x - 0.046. Data were log transformed to retain normality. Enzyme samples contained β-Glucuronidase / Arylsulfatase and Non-enzyme samples did not contain the enzyme.

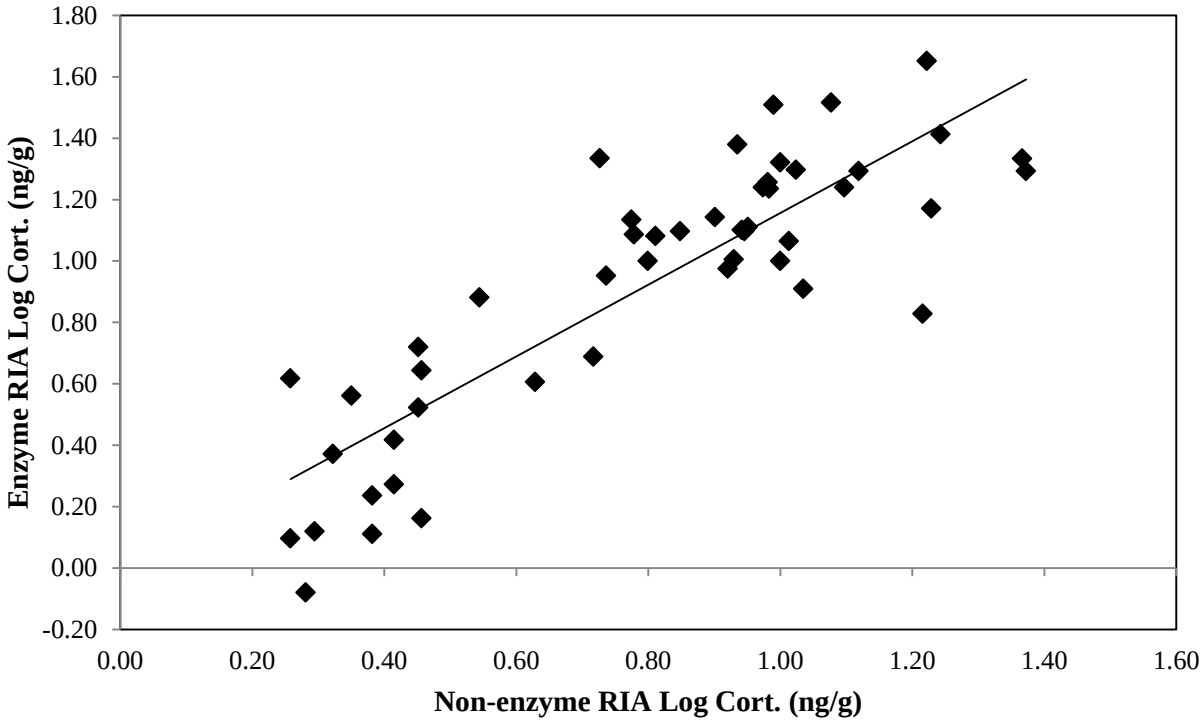


Figure 3.4. Relationship between fecal corticosterone concentrations with and without enzyme analyzed using radioimmunoassay (RIA). Corticosterone is expressed in ng of hormone per g of dry feces of Sandhill Cranes (*Grus canadensis*) collected in Nebraska during March and April of 2009. Pearson's correlation coefficient = 0.86; y = 1.1675x - 0.0106. Data were log transformed to retain normality. Enzyme samples contained β-Glucuronidase / Arylsulfatase and Non-enzyme samples did not contain the enzyme.

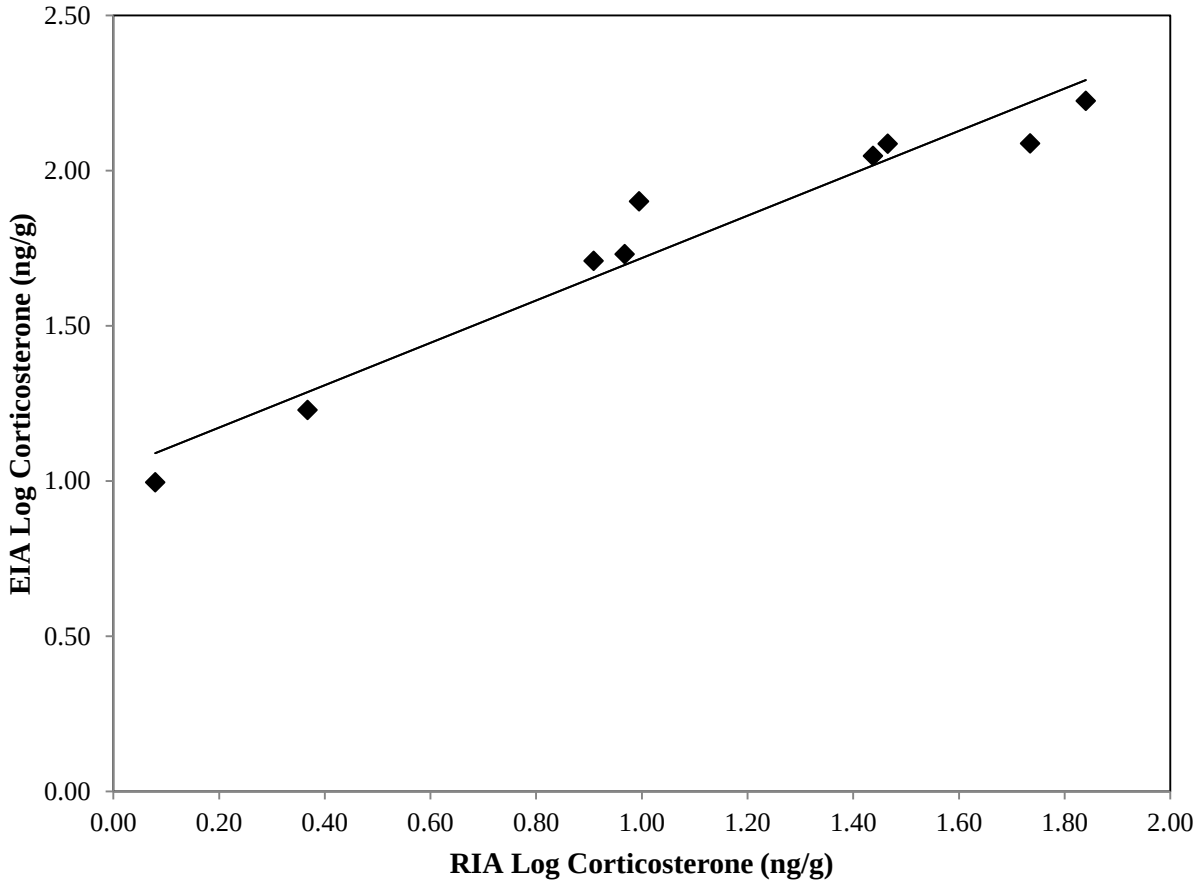


Figure 3.5. Relationship between fecal corticosterone concentrations analyzed with enzyme immunoassay (EIA) and radioimmunoassay (RIA). Corticosterone is expressed in ng of hormone per g of dry feces of Sandhill Cranes (*Grus canadensis*) collected in Wisconsin between June and November of 2008. Pearson's correlation coefficient = 0.97; y = 0.682x + 1.0361. Data were log transformed to retain normality. Samples analyzed with EIA followed the kit instructions and did not have a custom-made enzyme added. Samples analyzed with RIA had β-Glucuronidase / Arylsulfatase enzyme added.

# CHAPTER 4: LIMITED RESOURCES IN THE CHIHUAHUAN DESERT INFLUENCE FOOD CHOICES BY WINTERING SANDHILL CRANES

## ABSTRACT

Sandhill Crane (*Grus canadensis*) diet consists mainly of corn and other agricultural products, but also invertebrates and small vertebrates. However, most diet studies have focused on their feeding habits during migration and breeding and few have examined diet during the winter. I investigated crane diet in northern Mexico during the winters of 2007 and 2008. I quantified food items consumed based on an analysis of feces, examined the effects of agriculture on diet, and compared my results with studies in the United States and Canada to provide a more comprehensive picture of crane diet. I collected 320 fecal samples from six wetlands. The most commonly occurring food item was corn (47% of samples), followed by oats (22% of samples), and sorghum (22% of samples). Cranes also occasionally consumed grassland seeds (6% of samples), alfalfa (2% of samples), and wheat (<1% of samples). Diet in wetlands surrounded by agricultural fields did not differ from diet in wetlands surrounded by non-agricultural fields. My results suggest that cranes exhibit a specialized diet of corn and other agricultural products during winter as patterns of consumption did not vary with availability. My results differ from studies elsewhere, which suggest that cranes are opportunistic and omnivorous.

## INTRODUCTION

Diet of Sandhill Cranes has been well studied in their breeding and staging grounds both in the United States and Canada (Iverson et al. 1982, Krapu et al. 1984, Tacha et al. 1985, Reinecke and Krapu 1986). Although some studies have also looked at

diet in the wintering grounds in southern United States (Guthery 1975, Iverson et al. 1982, Walker and Schemnitz 1987, Hunt and Slack 1989, Ballard and Thompson 2000) all but one have concentrated in the state of Texas, probably because different parts of Texas offer diverse wintering habitats for the species. Indeed, most of the Mid-continent Population of Sandhill Cranes migrates to the state of Texas - providing winter habitat for 80% of the population (Krapu et al. 2011), although the remaining migrate to New Mexico, Arizona, and northern Mexico. Although it is estimated that around 14% of the population winters in northern Mexico (Drewien et al. 1996, Krapu et al. 2011), diet has never been investigated in this region where environmental conditions differ from the rest of the wintering grounds.

Cranes wintering in Mexico spend about five months of the year in a very arid region known as the Chihuahuan Desert with a mean annual rainfall between 125 and 400 mm (Ferrusquia-Villafranca et al. 2005). Despite its arid condition, the Chihuahuan Desert has been identified as one of the most important ecoregions in the world (Olson and Dinerstein 1998) with unique biological communities and specialized habitats (Dinerstein et al. 2001). Although the Chihuahuan Desert may be a low productivity ecosystem, there is no doubt that it has the capacity of providing food resources for large groups of cranes for extended periods of time. Food requirements for cranes should differ greatly from those calculated during the staging and breeding periods (Krapu et al. 1985, Reinecke and Krapu 1986), but dietary intake should at least provide for maintenance requirements to survive the winter months. Sandhill cranes stage in Nebraska during the spring migration for 4-6 weeks while the birds are known to increase their body weight up to 34% (Krapu et al. 1985). This weight gain is mostly due to a

substantial fat storage in a short period of time. It has been hypothesized that such an acquisition of fat reserves during spring migration has evolved as a mechanism to cope with both uncertain foraging conditions on breeding grounds and limited food resources on wintering grounds (Krapu et al. 1985). Therefore, wintering habitat in Mexico may represent a maintenance-type environment that allows cranes to sustain body weight until they reach the staging grounds.

Previous studies have determined that Sandhill Cranes are omnivorous and opportunistic birds feeding on a variety of plant and animal matter (Nelson 1887, Harvey et al. 1968, Guthery 1976, Mullins and Bizeau 1978, Reinecke and Krapu 1986, Meine and Archibald 1996). The diets described in those studies would also classify cranes as generalists – feeding indiscriminately from a wide range of food items (Belovsky 1978, Roper 1994). However, most of these studies were performed during summer or spring when cranes are known to become more omnivorous, ingesting invertebrates in order to obtain a supplement of calcium and protein in preparation for the breeding season (Reinecke and Krapu 1986). Other species of cranes exhibit similar behavior, including Common Cranes (*G. grus*) in Spain, which feed on animal matter during the summer and plant material for the remainder of the year (Aviles et al. 2002). Specifically, diet studies of Sandhill Cranes have concluded that the species feeds mainly on agricultural grains (Ballard and Thompson 2000), noncultivated food types (Hunt and Slack 1989), invertebrates (Guthery 1975), and even small vertebrates (Harvey et al. 1968).

Winter distribution of the species in Mexico occupies a large geographical area with different groups of cranes having access to different food resources. The 'profitability' theory suggests that animals will feed in areas where their success rate is

highest (Royama 1970). In areas where their preferred food type is less available, foragers will reach a point when the energetic cost of searching for that food is balanced by its energy content and no longer profitable. However, the theory predicts that before this point of non-profitability is reached the animal will either move to a new area with greater food density or, alternatively, switch to a different food item within its current area (Royama 1970). Alonso et al. (1994) found that the number of wintering Common Cranes correlated with food availability in the area; furthermore, the population was limited by the carrying capacity of the area which influenced how many birds migrated further south.

I investigated winter diet of Sandhill Cranes in northern Mexico through fecal samples. The goal of this study was to compare diets between sites with different food availability (i.e., high, medium, and low). Diet similarities between sites were quantified through niche overlap (MacArthur and Levins 1967) based on the relative utilization of different resource components and the amount of overlap in the use of those components. Although the concept of niche overlap was developed to investigate competition (MacArthur and Levins 1967) and ecological character displacement (Bulmer 1974) it has also been used to quantify resource utilization in the absence of competition (Connell 1980). My null hypothesis was that diet composition varied among sites (i.e., wetlands), so that any similarity observed was due only to chance. Alternatively, I proposed that the type of wetland would cause no differences in the composition of crane diet.

The objectives were to (1) determine the ecological response of Sandhill Cranes wintering in low food availability sites according to the 'profitability' theory, by investigating if cranes moved geographically to a location with food or they shifted to a

different and more available source of energy (diet shift); (2) determine if Sandhill Cranes exhibit a specialized or generalized diet during winter by quantifying diet diversity through niche breadth; (3) assess Sandhill Crane diet under the conditions of the 'food specialist' theory, by determining if cranes rely to a disproportionate extent on a single type of food and if the consumption of this predominant food is independent of its availability; and (4) compare Sandhill Crane diet in Mexico with previous winter food studies.

## MATERIALS AND METHODS

### *Study Area*

I sampled the diet of Sandhill Cranes in wetlands distributed within the wintering range of Sandhill Cranes in northern Mexico in the Chihuahuan Desert Ecoregion (Dinerstein et al. 2001) (Fig. 4.1). The region covers 629,000 $km^2$ and stretches north-south from south-central United States to central Mexico, where it includes a portion of the state of Chihuahua, most of Coahuila, eastern Durango, northern Zacatecas, northern and central San Luis Potosí, and some small portions of Nuevo Leon and Tamaulipas (Fig. 4.1). The Chihuahuan Desert is bordered by the Sierra Madre Occidental to the west and the Sierra Madre Oriental to the east.

Wetlands represent critical habitat for cranes; cranes use wetlands as roost sites during the night and rest sites during the hottest hours of the day. Therefore, I collected my samples from wetlands historically used by cranes. I selected six wetlands to represent three contrasting habitat conditions for wintering cranes with regards to proximity and abundance of food resources. My study sites spanned the distribution of

cranes in the Chihuahuan Desert Ecoregion: (1) Laguna de Babícora (state of Chihuahua; Fig. 4.2), and (2) Laguna de Mexicanos (state of Chihuahua; Fig. 4.3) surrounded by abundant food resources; (3) Laguna de Ojo Federico (state of Chihuahua; Fig. 4.4), and (4) Laguna Victorio (state of Chihuahua; Fig. 4.5) relatively close to sparse food resources; and (5) Presa San Carlos de Mapimí (state of Durango; Fig. 4.6), and (6) Laguna de San Juan de Ahorcados (state of Zacatecas; Fig. 4.7) far from few food resources (Table 4.1).

*Data Collection*

I collected samples of feces of Sandhill Cranes from roost sites around the six wetlands described above during the winter (October to February) of 2007/08 and 2008/09. I located roost sites by direct observation of flocks of cranes leaving at dawn. I identified samples and placed them in individual and sterile plastic whirl-pak bags (Nasco Whirl-Pak, Fort Atkinson, Wisconsin); numbered, dated, and assigned a location name for each one. I stored the samples in a freezer at the Laboratorio de Transgenesis Animal y Fertilización In Vitro of the Universidad Autónoma de Chihuahua for better preservation and to avoid fungal development until processing.

I used spectral and spatial analyses of satellite images to estimate the area of cropland to indicate the amount of food resources available to cranes around each wetland (Jensen 2005). I downloaded current images from the Earth Science Data Interface (ESDI 2012) version 2.1.17 web application (Global Land Cover Facility, University of Maryland, College Park, Maryland) (Table 4.2). I used ArcGIS version 9.3 software (Environmental Systems Research Institute, Redlands, California) to process the images. I used Composite Bands from the Data Management ArcToolbox in ArcMap

(ArcGIS) to create a single raster dataset from multiple bands. I used combinations of spectra typically used for these types of delineations. The best spectral band combination for the delineation of the wetland was 3, 2, and 1 (red: 0.63-0.69 µm, green: 0.52-0.60 µm, and blue: 0.45-0.51 µm, wavelengths respectively; Sheffield 1985). This is a natural color band combination with ground features appearing in colors similar to their appearance to the human eye. The best spectral band combination to represent agricultural fields was 4, 3, and 2 (near infrared: 0.75-0.90 µm, red: 0.63-0.69 µm, and green: 0.52-0.60 µm, wavelengths respectively; Sheffield 1985). This is a standard false color composite with ground features appearing in colors similar to traditional infrared aerial photography. With this combination of spectra, vegetation and farmland appeared in shades of red; such an approach is used in studies of crop growth because areas in red are easily distinguished as productive agricultural fields (Jensen 2005). I projected the images using the World Geodetic System (WGS) 1984 datum and Universal Transverse Mercator (UTM) geographic coordinate system.

I used the landscape data obtained from the satellite images to categorize the six wetlands into high, medium, and low food abundance for Sandhill Cranes (Fig. 4.8-4.13). To verify that the types of crops identified from the satellite images corresponded to crop types that cranes were able to consume, I obtained official records from a government data base, the Sistema Estatal y Municipal de Bases de Datos, on the sown area of major crops (per Mexican government: corn, oats, sorghum, alfalfa, wheat, beans, green chili, red tomatoes, and pastures) in each municipality (SIMBAD 2012; Fig. 4.14).

I created a GIS layer of polygons to determine the area occupied by each wetland in the satellite images. Wetlands in the Chihuahuan Desert Ecoregion tend to have

variable water levels; wetlands fill to maximum capacity during the rainy season of July, August, and September. Wetlands are prone to evaporation, and thus reduce in size, during the dry season between October and June. In addition, some wetlands used by cranes are temporary and can dry completely by the end of winter. During dry periods when wetland water evaporates, crystallized salts form on the shores leaving white broad lines that can be seen from the satellite images. I used a series of historical images to find the extension of the minimum and maximum wetland sizes during recent time periods (i.e., previous 5-8 years) for each wetland. I created a 20-km buffer zone around the maximum wetland size polygon to delimit the foraging area potentially used by cranes (Iverson et al. 1985). Food abundance within a 20-km radius of the roost is one of the main environmental variables that influences crane distribution in winter grounds (Iverson et al. 1985); however, the maximum distance that cranes have been recorded to disperse from their roosting sites in search for food is 13 km (Iverson et al. 1985). I then created a GIS layer of polygons to delineate the area of cropland inside the buffer zone. For each wetland, therefore, I obtained the total area dedicated to agriculture.

*Data Analysis*

Diet analyses.—In the laboratory, I processed the fecal samples using a Petri dish and a pair of thin tweezers to separate all the components. Because some of the samples were dry after being frozen for some time, I added distilled water to moisture and help break them easier. I examined the samples under a stereoscopic microscope and separated every seed and bran – the hard outer layer of a kernel that includes the pericarp and aleuron layer (Berghoff 1998), from the pulp or fecal mass. The rest of the fecal material that was too digested or small to be identified was discarded. When pulp and

seeds occurred together in the sample, I only recorded the seeds. For each sample, I counted and grouped seeds in operational taxonomic units (i.e., seeds considered as belonging to the same species based on morphological similarity) (Duraes and Marini 2005). I divided seeds into grains (domesticated seeds) and wild seeds. I identified grains with the use of a reference collection of the cultivated crops in the region; although grasses, sedges, and other seeds were identified by a specialist from the Universidad Autónoma de Chihuahua. Therefore, I divided food items found in feces into seven categories which included five categories of grains (corn, oats, wheat, sorghum, and alfalfa), wild seeds, and achenium fruits (i.e., simple dry fruit containing a single seed) (Rodford et al. 1976). I obtained information regarding the nature of the species identified from wild seeds (e.g., native, introduced, and/or invasive) from databases on taxonomy (ITIS 2012) and invasive species of Mexico (CONABIO 2011).

I described overall diet by estimating the frequency of occurrence of different food types in each sample of feces. I calculated percent occurrence as 100 times the number of occurrences of a particular food item in a feces sample, divided by the total number of occurrences of all food items in the sample. I used frequency of occurrence to quantify variability of prey abundance after Ashmole and Ashmole (1967) and MacDonald and Green (1983). Although volume of items in a sample may also be used (Duffy and Jackson 1986), frequency of occurrence is the most appropriate method when only a few food items are consumed by the species under study and those food items have similar size. I did not use percent volume, as calculating the volume of food items in bird fecal samples was not always possible due to the grinding action of the gizzard. The gizzard of grain and seed eaters such as cranes, is larger and more muscular allowing an

almost complete digestion of grains (Gill 1999). Frequency of occurrence is therefore the best method for diet studies in birds using fecal samples that are well digested (Duffy and Jackson 1986).

Statistical analyses.—I used rarefaction to compare differences in diet between groups of cranes wintering in contrasting habitat conditions and seasons (EcoSim Version 7.72; Gotelli and Entsminger 2011). Rarefaction uses probability theory to derive an expected number of species and its variance for a sample of a given size (Hurlbert 1971). EcoSim uses a Fisher's *F* test to calculate the overall probability level and yields an overall chi-square value based on null Monte Carlo distributions (Winemiller and Pianka 1990a). According to this principal, patterns in the data do not reflect biological forces but represent chance variation or sampling effects (Gotelli and Graves 1996). Therefore, any observed similarities in diet composition among wetlands would be due only to chance.

I used randomization algorithms (Winemiller and Pianka 1990b), housed in the module Niche Overlap in EcoSim, to investigate diet similarity between the three groups of cranes wintering in wetlands with high, medium, and low agricultural resources. To adapt the software for my purposes, I treated cranes from different wetland groups as different "species". I structured my data as count data (including zeros to represent food items that were not utilized by a particular group of cranes). The analysis is based on the concept of niche overlap as defined by MacArthur and Levins (1967) that quantifies the relative utilization of a niche resource axis between pairs of species. Although there are infinite number of resource axes that could be partitioned between species, diet is among the three most important niche axes (Schoener 1974). I created 1,000 random

assemblages (seed number: 0) of diet components, which I used to compare to my observed assemblage. I set my level of significance as $p \leq 0.05$, which corresponded to a decision to reject the null hypothesis if the observed values were less than or equal to 50 of the 1,000 values generated.

I measured niche overlap using the Pianka Index (Pianka 1973), in which species 1 and 2 have a resource utilization of $p_{1i}$ and $p_{2i}$ respectively, and the index of species 1 on species 2 ($O_{12}$) is calculated as follows:

$$O_{12} = O_{21} = \frac{\sum_{i=1}^{x} p_{2i}\, p_{1i}}{\sqrt{\sum_{i=1}^{x} \left(p_{2i}^{\,2}\right)\left(p_{1i}^{\,2}\right)}}$$

Pianka's Index is expressed on a scale from 0 to 1.0, where 0 indicates no overlap at all and 1 indicates a complete overlap.

The analysis of niche overlap uses four possible randomization algorithms (RA; Winemiller and Pianka 1990b) that the software allows you to specify. I used the default RA3 which uses the niche breadth as retained for each group of cranes by randomizing the utilization values within each row of the matrix. This means that the resources were used randomly in the null assemblages but the degree of specialization of each group of cranes was preserved. The other particularity of RA3 is that it allows for the zero states to reshuffle so that if a food type was not used by a group of cranes in reality, it could still be used in the null assemblages.

In addition to this analysis, I estimated diet diversity of each group of cranes per season and overall using the Index of Levins (Levins 1968, Krebs 1998). Levins'Index is used to estimate niche breadth by measuring the uniformity of distribution of individuals among the resource states. By estimating niche breadth I obtained a quantitative measure of diet specialization between groups. I calculated the dietary niche breadth (*B*) of each group of cranes as follows:

$$B = \frac{1}{\sum p_j^2}$$

where $p_j$ represents the proportion of food group *j* in the diet of cranes *p*. Breadth scores potentially ranged from one (only one food item consumed) to *n* (where *n* = number of food groups; all food groups consumed in equal proportions). *B* is maximum when an equal number of individuals occur in each resource state, so that the species does not discriminate among the resource states and has the broadest possible niche. Levins' *B* is minimal when all the individuals occur in only one resource state (minimum niche breadth, maximum specialization).

It is useful to standardize niche breadth to express it on a scale from 0 to 1.0. This can be done easily for Levins' measure by dividing *B* by the total number of resource states after correcting for a finite number of resources. Hurlbert (1978) suggested the following measure for standardized niche breadth:

$$B_A = \frac{B-1}{n-1}$$

where:

$B_A$ = Levins' standardized niche breadth

$B$ = Levins' measure of niche breadth

$n$ = Number of possible resource states

**RESULTS**

Lagunas de Babícora and Mexicanos had >500 km$^2$ of cropland inside the crane's foraging area. In contrast, Laguna de San Juan de Ahorcados and Presa San Carlos de Mapimí had <100 km$^2$ of cropland área (Table 4.3). Gómez Farías, the municipality where Laguna de Babícora is located, had the highest percentage of planted corn (74%) and Cusihuiriachi, the municipality where Laguna de Mexicanos is located, was the area with the highest percentage of oats (35%). The municipalities that host the wetlands with less cropland area, Tlahualilo – home to Presa San Carlos de Mapimí, and General Francisco R. Murguía – home to Laguna de San Juan de Ahorcados, planted beans (57%) and alfalfa (21%) as major crops (Fig. 4.14). On the other hand, Chihuahua was the state with the highest surface of available grassland (Table 4.6) which decreases as we move south.

I collected 320 fecal samples in all study sites during the winters of 2007/08 ($n$ = 147) and 2008/09 ($n$ = 173). The most abundant food item was corn (*Zea mays*, 51% of samples, $n$ = 178), followed by oats (*Avena sativa*, 19%, $n$ = 65) and sorghum (*Sorghum bicolor*, 18%, $n$ = 64). Seeds from non-domesticated species were found in 9% ($n$ = 30) of the samples. Other food items such as alfalfa (*Medicago sativa*, 2%, $n$ = 6), wheat (*Triticum aestivum*, 1%, $n$ = 3), and fruits (1%, $n$ = 2) were found in few samples.

Seeds from eight species of wild plants were identified to at least family level (Table 4.4). Four species were seeds of grassland origin, including three *true grasses* belonging to the family Poaceae and one sedge belonging to the family Cyperaceae. Half of these grassland species are native to Mexico and the other half have been introduced and are also considered invasive (Appendix F). None of the native species are endangered or subject to special protection by the Mexican government (SEMARNAT 2010). The only fruit found in the samples were asters belonging to the genus *Helenium*.

I found that overall winter diets were similar between years (mean pairwise niche overlap = 0.961; $P < 0.05$) (Fig. 4.15). Samples collected in the two wetlands with high food resources ($n = 151$) contained 172 food items, samples from the two wetlands with medium food resources ($n = 77$) contained 78 items, and finally samples from the remaining wetlands with low food resources ($n = 92$) accounted for 98 food items (Fig. 4.16). The diversity of cranes' diet did not differ among the three levels of cropland availability (mean pairwise niche overlap = 0.862; $P < 0.05$) (Fig. 4.17), indicating that crane diets overlapped significantly more than expected by chance (Table 4.5).

Niche breadth for cranes wintering in wetlands with high, medium, and low proportion of cropland (food resources) in their foraging areas were $B = 2.928$, $B = 3.403$, and $B = 2.159$, respectively. Thus, only two or three food items were found in most samples suggesting that most individuals accessed three or fewer resource states. Such small niche breadths indicate that cranes discriminated among other available food resources suggesting a strong degree of specialization. Standardized niche breadth for cranes wintering in wetlands with high, medium, and low food resources were $B_A = 0.321$, $B_A = 0.400$, and $B_A = 0.193$, respectively. These suggest that cranes wintering in

wetlands with medium crop availability tended to have a more diverse diet than cranes with access to more food resources, although cranes wintering in wetlands with low crop availability tended to have the least diverse diet.

**DISCUSSION**

This is the first study to quantify the winter diet of Sandhill Cranes in Mexico where a portion of the Mid-continent Population spends about five months of the year. I showed that their diet is mainly composed of corn and, despite differences in food abundance among wetlands used by cranes, their food choices were similar among different wetlands.

The results of my study confirmed that cranes wintering in Mexico fed mostly on agricultural grains coinciding with most previous studies. Diet of Sandhill Cranes is dominated by agricultural grains in North America (Ballard and Thompson 2000). In fact, Iverson et al. (1982) concluded that cereal grains accounted for 96% of the aggregate volume of crane diet along their distribution range between Texas and Alaska. In Saskatchewan, grains constituted 100% of foods consumed during autumn migration (Tacha et al. 1985). Similar results were reported from Nebraska (Krapu et al. 1984, Reinecke and Krapu 1986) where cranes fed extensively on waste grains (97% total dry weight) during spring migration. In Texas, where most Sandhill Crane winter diet studies have been done, agricultural grains were also reported to compose up to 89% of their diet (Ballard and Thompson 2000). In New Mexico and Arizona, grains were also the main food resource for cranes (Perkins and Brown 1981, Walker and Schemnitz 1987). However, other studies have showed the importance of noncultivated food types, including native plants and animal matter, in crane diet with up to 87-99% frequency of

occurrence in areas of Texas dominated by prairie and brushland (Guthery 1975, Hunt and Slack 1989).

Crops consumed by cranes in the Chihuahuan Desert were corn, oats, and sorghum. These results compared to studies conducted in the wintering grounds (from Texas to Arizona), were only similar with diet of cranes in New Mexico where corn represented 71% frequency of occurrence (Walker and Schemnitz 1987). For the rest of existing wintering studies, the results were not similar. For instance, in western and lower Gulf coast of Texas cranes relied on sorghum as their primary food resource (Iverson et al. 1982, Ballard and Thompson 2000), whereas wheat was the predominant grain in the northern and southern plains, and rice and corn were important in the middle Gulf coast (Ballard and Thompson 2000). These contrasting results point out that cranes feed on the grains that are mostly available in each wintering area and emphasizes the variation in diet among habitats as it had previously been described by Reinecke and Krapu (1986). In my study area, since corn and oats were the predominant crops, it was expected that those would be the main grains consumed by cranes.

Wetlands used by Sandhill Cranes along their distribution range in Mexico had differences in terms of crop availability within the delineated buffer zone. Wetlands in the south had less cropland than wetlands in the north. The greater availability of food surrounding northern wetlands could explain why more cranes concentrate in the northern states of Chihuahua and Durango than in any other state in Mexico (Drewien et al. 1996, Perez-Arteaga et al. 2005, Lopez-Saut et al. 2011). Similar results were found in areas of Spain where Common Cranes shifted their winter range to adjust it to areas with increasing agricultural food resources (Alonso et al. 1994). According to the Ideal

Free Distribution theory (Fretwell and Lucas 1970, Fretwell 1972) the distribution of organisms between different resource sites should match the distribution of resources. Historically, cranes used to winter as far south as the states of Puebla and Yucatan (Leopold 1965) but changes in agricultural practices and habitat loss during the last four decades have re-shaped the distribution range of the species in Mexico. More recently, survey studies have updated the distribution of cranes recovering some historical sites and adding new ones in the states of Zacatecas and San Luis Potosi, probably due to an increase in agriculture in these states (Lopez-Saut et al. 2011). Although these locations in the south have less crop availability, they support a small proportion of cranes that migrate into the region in search of a few wintering sites. Wetlands in the north may reach carrying capacity and force later arriving cranes to displace further south. The 'ideal free distribution' theory predicts that the suitability of an area declines as the density of animals increases (Fretwell and Lucas 1970). Other bird species, including Common Cranes (Alonso et al. 1994), Eurasian Oystercatchers (*Haematopus ostralegus*) (Goss-Custard et al. 1992) and Herring Gulls (*Larus argentatus*) (Monaghan 1980), follow similar patterns of distribution when areas become overpopulated.

I showed that diets of cranes wintering in wetlands with low food resources managed to find food since diets did not vary between wetlands. Cranes responded to a low availability of corn by moving geographically to a location where they could find it instead of shifting diets. Although I did not collect data on body condition, these birds probably expended more energy to obtain their food by flying longer distances. Similar responses have been observed in other species of waterbirds including the Eurasian Oystercatcher (Heppleston 1971, O'Connor and Brown 1977), the Great Blue Heron

(*Ardea herodias*) (Krebs 1974), the Red Knot (*Calidris canutus*) (Prater 1972), and the Common Redshank (*Tringa totanus*) (Goss-Custard 1970). Cranes in the two wetlands with low crop abundance could have switched to an alternative food item - oats. The other two available crops in these wetlands were alfalfa and beans which have not been reported as main diet components for cranes. Though cranes are known to consume alfalfa in small proportions to obtain protein from emerging shoots (Reinecke and Krapu 1986), it does not contain essential carbohydrates to sustain their diets (Reinecke and Krapu 1986).

Although most authors have characterized Sandhill Cranes as omnivore and opportunist feeders (Nelson 1887, Harvey et al. 1968, Guthery 1976, Mullins and Bizeau 1978, Reinecke and Krapu 1986, Meine and Archibald 1996), the species is known to largely feed on corn during most of its life history. However, Ballard and Thompson (2000) stated that Sandhill Cranes in Texas are not limited to agricultural grains and that they will alter their foraging behavior to obtain high-energy native foods. My data provides evidence that wintering cranes are predominately herbivorous, which corresponds with other winter studies (Iverson et al. 1982, Walker and Schemnitz 1987, Ballard and Thompson 2000); but furthermore, my data suggests that wintering cranes in Mexico behave as feeding specialists – with a narrow range of food preferences, sometimes even for a single food item (Roper 1994, Sinclair et al. 2006). Their specialized behavior was supported by the results of similar diet diversity among the three groups of cranes. Niche breadth was small (consuming only 2 out of 8 possible food items) indicating that cranes discriminated among other available food resources and further suggesting a strong degree of specialization (Levins 1968, Krebs 1998). In

conclusion, diets among wetlands were similar in both composition and proportion of food items demonstrating that cranes have become advantageous specialists on corn (e.g. they still have the ability to be generalists).

According to the 'food specialist' hypothesis (Roper 1994) there are two conditions: 1) a population of a species relies to a disproportionate extent on a single type of food; and 2) consumption of the predominant food is independent of its availability. The first condition was not supported by the Mid-continent Population because cranes switch to other food types due to nutritional requirements during their life history. However, when examining only wintering cranes in northern Mexico, both conditions were supported by my data. For that reason, I believe that only cranes wintering in Mexico exhibit an advantageous specialization on corn.

Choosing a diet based on corn is advantageous to cranes because it is rich in carbohydrates and energy and it is obtainable throughout most of their life history. However, having such specialized diet has some associated consequences that impact this region. At present, cranes affect 43% of farmers in northern Mexico due to their feeding habits (Barcelo et al. 2012). Negative effects include destroyed crops with a subsequent diminished production; as a consequence, conflicts between humans and cranes are expected to rise in the future. If corn production persists in the region, it seems likely that cranes will also persist in agricultural regions and continue their population increasing trend (Krapu et al. 2011), with a consequent increase in corn demand. On the other hand, there are also risks associated with such dependency if corn production was to be reduced in the future. In fact, corn production in the region is already changing due to the impacts of highly subsidized corn producers in the United States and Canada that

outcompete small farming operations in northern Mexico (Koechlin and Larudee 1992). Climate change may also have an impact on corn production in the Chihuahuan Desert with theoretical expectations indicating a decrease in corn yields in the next 100 years (Galindo 2010). One of the proposed potential actions to compensate these impacts is the increase of water usage for corn production (Galindo 2010). However, this is a limited and non-sustainable procedure in the long term that would have other negative impacts for cranes roosting in the region. In the future, farmers may need to plant other more resilient types of crops or even abandon their farms. Sandhill Cranes have the capacity to exploit other food types; therefore, either of these options would force them to switch diet and become feeding generalists. The question remains if other foods would supply enough energy to sustain the increasing population demands of Sandhill Cranes.

Desert grasslands used to be the predominant ecosystem in this region of northern Mexico as an extension of the Great Plains, but have been lost to either shrub encroachment or converted to agriculture production (Askins et al. 2007). The percentage of grassland surface available to wildlife has been reduced up to 50% in some areas (Dinerstein et al. 2001). Chihuahua is the state with more and better preserved grasslands probably because most of them are privately owned by cattle ranches which help preserve prairies to some extent (Askins et al. 2007). The surface of available grasslands decreases as we move south which could be another reason, other than food availability, why more Sandhill Cranes are found in the state of Chihuahua than in any other Mexican state (Drewien et al. 1996, Krapu et al. 2011). Sandhill Cranes are considered grassland birds (Meine and Archibald 1996) even though at present their diet is mainly composed of agricultural grains, as described previously. In the past, cranes

used to feed on grassland seeds but have adapted their diet to benefit from a more nutritious and abundant form of food (Reinecke and Krapu 1986). This could explain why seeds represented only 9% of Sandhill Crane diet in winter. Although in less proportion (7%), Walker and Schemnitz (1987) also reported grassland seeds in the diet of cranes in New Mexico. These are the only two accounts of grassland seeds consumed by Sandhill Cranes during the winter season. On the other hand, cranes are known to use grassland habitat for other purposes such as resting (Krapu et al. 1984) or foraging for terrestrial invertebrates (Reinecke and Krapu 1979, 1986). Invertebrates were not present in the samples of my study suggesting that cranes used grasslands to obtain seeds; besides, invertebrates were not available during this time of the year (pers. observ.). Other winter diet studies have reported animal matter representing very small percentages and only present in areas where milder temperatures allow invertebrates to survive (Iverson et al. 1982). Invertebrates become an important food source during spring migration when birds prepare for the breading season (Reinecke and Krapu 1986) and may not be needed during winter when cranes are expected to seek more carbohydrates and energy in the form of grains in order to survive cold conditions.

Sandhill Cranes may contribute to the process of endozoochory by which animals serve as agents of seed dispersal through defecation (Van der Pijl 1972). Several studies have explored the importance of birds in seed dispersal and how it may benefit plant species (Krefting and Roe 1949, Malmborg and Willson 1988, White and Stiles 1990). Seeds consumed by birds may increase their chances of colonizing further and favorable sites for plant regeneration. In the same way, birds can be responsible for the dispersal of exotic species of plants (Smith 1975). I suggest that cranes could be contributing to the

dispersal of exotic species in the Chihuahuan Desert Ecoregion because two of the grassland seeds found in the samples I collected were introduced and invasive species in Mexico. Although cranes have a well-developed and strong gizzard (Gill 1999), most of the seeds were found complete suggesting they could be viable for germination. In fact, some species of plants require scarification of their seeds as they pass through the digestive tract of birds in order to break seed dormancy (Krefting and Roe 1949). Mechanical scarification is the process of breaking down the impermeability of a seed by cutting or softening the external hard coat (Blazich and Evans 1999). This process is achieved through mechanical grinding in the gizzard of some birds (Traveset et al. 2001). Plant species adapted to desert environments that depend on rainy seasons to germinate can benefit from scarification (Traveset et al. 2001). Although both invasive species of grasslands are already widespread and established in the area, they are not considered species of special concern by the Mexican authorities (CONABIO 2011).

Diet was examined through the analysis of fecal samples. The amount of samples collected and analyzed was adequate for this kind of studies (Trites and Joy 2005). Although this technique provides a non-invasive approach to diet studies and allows for a bigger sample size, it makes identification of food items more problematic than studies that examine esophagus or gizzard contents of dead individuals. Additionally, the remains of food items identifiable in gizzard and feces may be biased towards some foods (Swanson and Bartonek 1970, Ballard and Thompson 2000) because different food items have different digestibility which may affect their relative proportion in feces (Swanson 1940). It is possible therefore, that my results could be biased towards some agricultural grains that were less digested than others. However, previous controlled experiments in

birds have suggested that most food items produce identifiable remains in feces (Jensen and Korschgen 1947). On the other hand, the use of different methods and units in previous studies of crane food habits complicates comparison between results. Only one other winter diet study used fecal analyses (Hunt and Slack 1989) and found 8 food items versus 7 food items present in my study. The fact that this other study found the presence of very different food contents (e.g., wolfberry fruits, acorns, and insects) suggests that fecal analyses are capable of identifying a wide arrange of potential foods consumed by cranes.

Estimating the number of cranes present at each wetland could have provided a better assessment of food resources availability and a better estimate of whether food resources were low in relation to crane needs. However, I did not collect systematic crane counts for all of the wetlands included in the study to make it a reliable estimate of crane abundance. Furthermore, results obtained from calculating the number of cranes per area of cropland could indicate an adequate amount of food resources in every site because there are already less cranes in those areas where food is scarce coinciding with the Ideal Free Distribution theory (Fretwell and Lucas 1970, Fretwell 1972).

Future research should focus on better understanding wintering site selection and site fidelity by cranes in this region of Mexico. Wetland use by Sandhill Cranes may be determined by fresh water availability (Iverson et al. 1985a) and access to undisturbed roosting sites (Lovvorn and Kirkpatrick 1981) rather than proximity to food resources. However, cranes in Europe stopped using a historical staging area where agriculture had ceased even though fresh water resources were still available (G. Krapu, pers. comm.). Wetland selection by cranes in Mexico may follow a different pattern where the energetic

cost of flying to distant agricultural fields may be offset by remoteness and safety. Having a better knowledge on how the species selects wintering wetlands will help in determining priority conservation efforts.

Sandhill Cranes can feed on a diverse array of agricultural grains; however, they have developed a favorite for a particular crop. Cranes have become advantageous specialists on corn maintaining a clear preference for this crop as patterns of consumption did not vary with availability, yet they still have the capacity to become generalists during other periods of their life history. Cranes will expend more energy in order to find corn rather than switching to other food items with less energy contain such as oats. The advantage of feeding on a high caloric food type provided by humans in great quantities in most of their distribution range offsets the costs of obtaining it in areas where it is scarce.

**LITERATURE CITED**

Alonso, J. C., J. A. Alonso, and L. M. Bautista. 1994. Carrying capacity of staging areas and facultative migration extension in common cranes. Journal of Applied Ecology 31:212-222.

Ashmole, N. P., and M. J. Ashmole. 1967. Comparative feeding ecology of seabirds of a tropical oceanic island. Bulletin of the Peabody Museum of Natural History 24.

Askins, R. A., F. Chavez-Ramirez, B. C. Dale, C. A. Haas, J. R. Herkert, F. L. Knopf, and P. D. Vickery. 2007. Conservation of grassland birds in North America:

Understanding ecological processes in different regions. Ornithological Monographs 64:1-46.

Aviles, J. M., J. M. Sanchez, and D. Parejo. 2002. Food selection of wintering common cranes (*Grus grus*) in holm oak (*Quercus ilex*) dehesas in south-west Spain in a rainy season. Journal of Zoology 256:71-79.

Ballard, B. M., and J. E. Thompson. 2000. Winter diets of sandhill cranes from central and coastal Texas. The Wilson Bulletin 112:263-268.

Barcelo, I., J. C. Guzman-Aranda, F. Chavez-Ramirez, and L. A. Powell. 2012. Rural inhabitant perceptions of sandhill cranes in wintering areas of northern Mexico. Human Dimensions of Wildlife. In press.

Belovsky, G. E. 1978. Diet optimization in a generalist herbivore: the moose. Theoretical Population Biology 14:105-134.

Berghoff, W. 1998. Längsschnitt durch ein Getreidekorn. Aid infodienst (A wheat kernel and its nutritional value. Data sources). Verbraucherschutz Ernährung, Bonn, Germany.

Blazich, F. A., and E. Evans. 1999. Overcoming seed dormancy: trees and shrubs. North Carolina Cooperative Extension Service 1/99 HIL-8704.

Bulmer, M. G. 1974. Density-dependent selection and character displacement. The American Naturalist 108:45-58.

CONABIO. 2011. Sistema de información sobre especies invasoras en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México. [http://www.conabio.gob.mx/invasoras].

Connell, J. H. 1980. Diversity and the coevolution of competitors, or the ghost of competition past. Oikos 35:131-138.

Dinerstein, E., D. Olson, J. Atchley, C. Loucks, S. Contreras-Balderas, R. Abell, E. Iñigo, E. Enkerlin, C. Williams, and G. Castilleja. 2001. Ecoregion-Based Conservation in the Chihuahuan Desert: A Biological Assessment. WWF, CONABIO, TNC, PRONATURA Noreste, and ITESM.

Drewien, R. C., W. M. Brown, and D. S. Benning. 1996. Distribution and abundace of sandhill cranes in Mexico. Journal of Wildlife Management 60:270-285.

Duffy, D. C., and S. Jackson. 1986. Diet studies of seabirds: a review of methods. Colonial Waterbirds 9:1-17.

Duraes, R., and M. A. Marini. 2005. A quantitative assessment of bird diets in the Brazilian Atlantic forest, with recommendations for future diet studies. Ornitologia Neotropical 16:65-83.

ESDI. 2012. Earth Science Data Interface. Global Land Cover Facility. University of Maryland, College Park, Maryland. [http://glcfapp.glcf.umd.edu:8080/esdi/index.jsp].

Ferrusquia-Villafranca, I., L. I. Gonzalez, and J. E. Cartron. 2005. Northern Mexico's landscape, part I: the physical setting and constraints on modeling biotic evolution Pages

11-28 *in* J. E. Cartron, G. Ceballos, and R. S. Felger, editors. Biodiversity, ecosystems, and conservation in northern Mexico. Oxford University Press, Inc., New York.

Fretwell, S. D. 1972. Populations in a seasonal environment. Princeton University Press, Princeton, NJ.

Fretwell, S. D., and J. H. L. Lucas. 1970. On territorial behaviour and other factors influencing habitat distribution in birds. Acta Biotheoretica 19:16-36.

Galindo, L. M. 2010. The Economics of Climate Change in Mexico: Synopsis. SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales), Mexico, D.F.

Gill, F. B. 1999. Ornithology. 2nd edition. W. H. Freeman and Company, New York City, New York, USA.

Goss-Custard, J. D. 1970. The responses of redshank (*Tringa totanus* (L.)) to spatial variations in the density of their prey. Journal of Animal Ecology 39:91-113.

Goss-Custard, J. D., R. W. G. Caldow, and R. T. Clarke. 1992. Correlates of the density of foraging oystercatchers *Haemotopus ostralegus* at different population sizes. Journal of Animal Ecology 61:159-173.

Gotelli, N. J., and G. L. Entsminger. 2011. EcoSim: Null models software for ecology. Version 7.72. Acquired Intelligence Inc. & Kesey-Bear. [http://homepages.together.net/~gentsmin/ecosim.htm].

Gotelli, N. J., and G. R. Graves. 1996. Null models in ecology. Smithsonian Institution Press, Washington, DC.

Guthery, F. S. 1975. Food habits of sandhill cranes in southern Texas. Journal of Wildlife Management 39:221-223.

Guthery, F. S. 1976. Foods and feeding habitat of sandhill cranes in southern Texas. Proceedings of the International Crane Workshop 1:117-125.

Harvey, J. M., B. C. Lieff, C. D. MacInnes, and J. P. Prevett. 1968. Observation on behavior of sandhill cranes. Wilson Bulletin 80:421-425.

Heppleston, P. B. 1971. The feeding ecology of oystercatchers (*Haematopus ostralegus L.*) in winter in northern Scotland. Journal of Animal Ecology 40:651-672.

Hunt, H. E., and R. D. Slack. 1989. Winter diets of whooping and sandhill cranes in south Texas. Journal of Wildlife Management 53:1150-1154.

Hurlbert, S. H. 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52:577-586.

Hurlbert, S. H. 1978. The measurement of niche overlap and some relatives. Ecology 59:67-77.

ITIS. 2012. Taxonomic report of plants of North America. Integrated Taxonomic Information System. [http://www.itis.gov/index.html].

Iverson, G. C., P. A. Vohs, and T. C. Tacha. 1982. Food contents of sandhill cranes during winter and spring. Pages 95-98 *in* Proceedings 1981 Crane Workshop. National Audubon Society, Grand Tetons National Park, Wyoming.

Iverson, G. C., P. A. Vohs, and T. C. Tacha. 1985a. Distribution and abundance of sandhill cranes wintering in western Texas. Journal of Wildlife Management 49:250-255.

Iverson, G. C., P. A. Vohs, and T. C. Tacha. 1985b. Habitat use by sandhill cranes wintering in western Texas. Journal of Wildlife Management 49:1074-1083.

Jensen, G. H., and L. H. Korschgen. 1947. Contents of crops, gizzards and droppings of bobwhite quail force-fed known kinds and quantities of seeds. Journal of Wildlife Management 11:37-43.

Jensen, J. R. 2005. Introductory digital image processing: a remote sensing perspective. 3rd. edition. Pearson Prentice Hall, Upper Saddle River, NJ.

Koechlin, T., and M. Larudee. 1992. The high cost of NAFTA. Challenge 35:19-26.

Krapu, G. L., D. A. Brandt, K. L. Jones, and D. H. Johnson. 2011. Geographic distribution of the Mid-Continent Population of sandhill cranes and related management applications Wildlife Monographs 175:1-38.

Krapu, G. L., D. E. Facey, E. K. Fritzell, and D. H. Johnson. 1984. Habitat use by migrant sandhill cranes in Nebraska. Journal of Wildlife Management 48:407-417.

Krapu, G. L., G. C. Iverson, K. J. Reinecke, and C. M. Boise. 1985. Fat deposition and usage by arctic-nesting sandhill cranes during spring. The Auk 102:362-368.

Krebs, C. 1998. Ecological methodology. 2nd edition. Addison-Welsey, New York City, New York, USA.

Krebs, J. R. 1974. Colonial nesting and social feeding as strategies for exploiting food resources in the Great Blue Heron (*Ardea herodias*). Behaviour 51:99-131.

Krefting, L. W., and E. I. Roe. 1949. The role of some birds and mammals in seed germination. Ecological Monographs 19:269-286.

Leopold, A. S. 1965. Fauna silvestre de Mexico: Aves y mamiferos de caza. Instituto Mexicono de Recursos Naturales Renovables, Mexico, D.F.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton, New Jersey, USA.

Lopez-Saut, E. G., F. Chavez-Ramirez, and R. Rodriguez-Estrella. 2011. New records of wintering grounds for sandhill cranes in Mexico. Waterbirds 34:239-246.

MacArthur, R., and R. Levins. 1967. The limiting similarity, convergence, and divergence of coexisting species. The American Naturalist 101:377-385.

MacDonald, J. C., and R. H. Green. 1983. Redundancy of variables used to describe importance of prey species in fish diets. Canadian Journal of Fisheries and Aquatic Sciences 40:635-637.

Malmborg, P. K., and M. F. Willson. 1988. Foraging ecology of avian frugivores and some consequences for seed dispersal in an Illinois woodlot. The Condor 90:173-186.

Meine, C. D., and G. W. Archibald. 1996. The cranes: Status survey and conservation action plan. IUCN, Gland, Switzerland, and Cambridge, U.K.

Monaghan, P. 1980. Dominance and dispersal between feeding sites in the Herring gull (*Larus argentatus*). Animal Behavior 28:521-527.

Mullins, W. H., and E. G. Bizeau. 1978. Summer foods of sandhill cranes in Idaho. The Auk 95:175-178.

Nelson, E. W. 1887. Natural history collection made in Alaska, 1877-1881. U.S. Army Signal Service, Arctic Series 3:19-230.

O'Connor, R. J., and R. A. Brown. 1977. Prey depletion and foraging strategy in the Oystercatcher *Haematopus ostralegus*. Oecologia 27:75-92.

Olson, D. M., and E. Dinerstein. 1998. The Global 200: a representation approach to conserving the Earth's most biologically valuable ecoregions. Conservation Biology 12:502-515.

Perez-Arteaga, A., S. F. Jackson, E. Carrera, and K. J. Gaston. 2005. Priority sites for wildfowl conservation in Mexico. Animal Conservation 8:41-50.

Perkins, D. L., and D. E. Brown. 1981. The sandhill crane in Arizona. Arizona Game and Fish Department Special Publication 11:1-47.

Pianka, E. R. 1973. The structure of lizard communities. Annual Review of the Ecology and Systematics 4:53-74.

Prater, A. J. 1972. The ecology of Morecambe Bay. III. The food and feeding habits of Knot (*Calidris canutus* L.) in Morecambe Bay. Journal of Applied Ecology 9:179-194.

Reinecke, K. J., and G. L. Krapu. 1979. Spring food habits of sandhill cranes in Nebraska. Proceedings 1978 Crane Workshop 2:13-19.

Reinecke, K. J., and G. L. Krapu. 1986. Feeding ecology of sandhill cranes during spring migration in Nebraska. Journal of Wildlife Management 50:71-79.

Rodford, A. E., W. C. Dickison, J. R. Massey, and C. R. Bell. 1976. Vascular plant systematics. Harper and Row, New York, NY.

Roper, T. J. 1994. The European badger *Meles meles*: food specialist or generalist? Journal of Zoology 234:437-452.

Royama, T. 1970. Factors governing the hunting behaviour and selection of food by the Great Tit (*Parus major L.*). Journal of Animal Ecology 39:619-668.

Schoener, T. W. 1974. Resource partitioning in ecological communities. Science 185:27-39.

SEMARNAT. 2010. Proteccion ambiental - Especies nativas de Mexico de flora y fauna silvestres - Categorias de riesgo y especificaciones para su inclusion, exclusion o cambio - Lista de especies en riesgo. Diario Oficial de la Federacion NOM-059-SEMARNAT-2010.

Sheffield, C. 1985. Selecting band combinations from multispectral data. Photogrammetic Engineering and Remote Sensing 51:681-687.

SIMBAD. 2012. Sistema Estatal y Municipal de Bases de Datos. INEGI. Mexico. [http://sc.inegi.org.mx/sistemas/cobdem].

Sinclair, A. R. E., J. M. Fryxell, and G. Caughley. 2006. Wildlife ecology, conservation, and management. 2nd edition. Blackwell Publishing, Malden, MA.

Smith, A. J. 1975. Invasion and ecesis of bird-disseminated woody plants in a temperate forest sere. Ecology 56:19-34.

Swanson, G. A. 1940. Food habits of the sharp-tailed grouse by analysis of droppings. Journal of Wildlife Management 4:432-436.

Swanson, G. A., and J. C. Bartonek. 1970. Bias associated with food analysis in gizzards of Blue-winged Teal. Journal of Wildlife Management 34:739-746.

Tacha, T. C., C. Jorgenson, and P. S. Taylor. 1985. Harvest, migration, and condition of sandhill cranes in Saskatchewan. Journal of Wildlife Management 49:476-480.

Traveset, A., N. Riera, and R. E. Mas. 2001. Passage through bird guts causes interspecific differences in seed germination characteristics. Functional Ecology 15:669-675.

Trites, A. W., and R. Joy. 2005. Dietary analysis from fecal samples: how many scats are enough? Journal of Mammalogy 86:704-712.

Van der Pijl, L. 1972. Principles of dispersal in higher plants. Springer-Verlag, New York, USA.

Walker, D. L., and S. D. Schemnitz. 1987. Food habits of sandhill cranes in relation to agriculture in central and southwestern New Mexico. Proceedings 1985 Crane Workshop 5:201-210.

White, D. W., and E. W. Stiles. 1990. Co-occurrences of foods in stomachs and feces of fruit-eating birds. The Condor 92:291-303.

Winemiller, K. O., and E. R. Pianka. 1990a. Organization in natural assemblages of desert lizards and tropical fishes. Ecological Monographs 60:27-55.

Winemiller, K. O., and E. R. Pianka. 1990b. Organization in natural assemblages of desert lizards and tropical fishes. Ecological Monographs 60:27-55.

Table 4.1. Geographic location of the wetlands included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Number | Wetland | Location | State | Municipality |
|---|---|---|---|---|
| 1 | Laguna de Ojo Federico | 31°2’57.21’’N 107°55’1.46’’W | Chihuahua | Ascensión |
| 2 | Laguna Victorio | 30°5’22.13’’N 107°13’34.48’’W | Chihuahua | Buenaventura |
| 3 | Laguna de Babícora | 29°21’46.64’’N 107°47’15.68’’W | Chihuahua | Gómez Farías |
| 4 | Laguna de Mexicanos | 28°10’36.44’’N 106°55’42.09’’W | Chihuahua | Cusihuiriachi |
| 5 | Presa San Carlos de Mapimí | 26°34’2.11’’N 103°44’49.56’’W | Durango | Tlahualilo |
| 6 | Laguna de San Juan de Ahorcados | 24°1’21.18’’N 102°17’48.14’’W | Zacatecas | General Francisco R. Murguía |

Table 4.2. Characteristics of satellite images downloaded from the Earth Science Data Interface (ESDI) used to estimate the area of agricultural land surrounding six wetlands included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Location | ID[a] | WRS: P/R[b] | Date Acquired | Sensor[c] | Producer[d] | Attributes[e] | Type[f] | Coordinate System[g] |
|---|---|---|---|---|---|---|---|---|
| Ojo Federico | 216-450 | 2: 034/038 | 10-21-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone12N |
| | 216-451 | 2: 034/039 | 10-21-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone12N |
| Babícora | 216-419 | 2: 033/040 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| Mexicanos | 216-419 | 2: 033/040 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| | 216-399 | 2: 032/040 | 10-10-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| | 216-420 | 2: 033/041 | 10-17-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| Victorio | 216-418 | 2: 033/039 | 09-28-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| San Carlos de Mapimí | 216-344 | 2: 030/042 | 10-28-2006 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |
| San Juan de Ahorcados | 216-320 | 2: 029/043 | 10-18-2005 | ETM+ | USGS | Ortho, GLS2005 | GeoTIFF | WGS_84 UTM_Zone13N |

[a] Landsat imagery online identification number.

[b] WRS = Worldwide Reference System, P = Path, R = Row.

[c] ETM+ = Enhanced Thematic Mapper Plus (provided by Landsat 7).

[d] USGS = United States Geological Survey.

[e] Ortho = Orthorectified, GLS2005 = Global Land Survey 2005.

[f] GeoTIFF = Geographic Tagged Image File Format.

[g] WGS_84 = World Geodetic System 1984, UTM = Universal Transverse Mercator.

Table 4.3. Wetland characteristics obtained from spectral and spatial analyses of satellite images to estimate area of agricultural land surrounding each wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. Food availability was determined by available croplands and was classified as high (cropland area >500 $km^2$), medium (500> cropland area >100 $km^2$), and low (cropland area <100 $km^2$).

| Wetland | State | Cropland Area ($km^2$) | Foraging Area ($km^2$) | Food Availability |
|---|---|---|---|---|
| Laguna de Babícora | Chihuahua | 800.6 | 2,788.4 | High |
| Laguna de Mexicanos | Chihuahua | 664.5 | 1,867.3 | High |
| Laguna de Ojo Federico | Chihuahua | 239.2 | 1,729.5 | Medium |
| Laguna Victorio | Chihuahua | 115.4 | 1,387.4 | Medium |
| Laguna de San Juan de Ahorcados | Zacatecas | 99.6 | 1,599.5 | Low |
| Presa San Carlos de Mapimí | Durango | 3.9 | 1,329.7 | Low |

Table 4.4. List of wild plants identified from seeds found in fecal samples of Sandhill Cranes (*Grus canadensis*) collected for a diet study in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Common Name | Order | Family | Genus | Species | Origin | Invasive |
|---|---|---|---|---|---|---|
| Plume thistle | Asterales | Asteraceae | *Cirsium* | — | — | — |
| Pondweeds | Charales | Characeae | — | — | — | — |
| Nut-grass | Cyperales | Cyperaceae* | *Cyperus* | *rotundus* | Introduced | Yes |
| Feather fingergrass | Cyperales | Poaceae* | *Chloris* | *virgata* | Native | No |
| Cockspur grass | Cyperales | Poaceae* | *Echinochloa* | *crus-galli* | Introduced | Yes |
| Sand dropseed | Cyperales | Poaceae* | *Sporobolus* | *cryptandrus* | Native | No |
| Silky sophora | Fabales | Fabaceae | *Sophora* | *nuttalliana* | Native | No |
| Moonflower | Solanales | Convolvulaceae | *Ipomoea* | — | — | — |

* Indicates a species of grassland origin.

Table 4.5. Pairwise niche overlap values (potential range: 0-1) based on food items found in fecal samples of Sandhill Cranes (*Grus canadensis*) collected from wetlands with different food resources availability for a diet study in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Wetland with Food Availability | High | Medium | Low |
|---|---|---|---|
| High | — | 0.837 | 0.983 |
| Medium | — | — | 0.766 |

Table 4.6. Percentage of grasslands surface available in each state where Sandhill Cranes (*Grus canadensis*) winter in northern Mexico. Data obtained from the Instituto Nacional de Estadística y Geografía (INEGI 2011).

| State | Political Area ($km^2$) | Grassland Area ($km^2$) | % Grasslands |
|---|---|---|---|
| Chihuahua | 247,455 | 45,833 | 18.52 |
| Durango | 123,451 | 14,004 | 11.34 |
| Zacatecas | 75,539 | 7,020 | 9.29 |

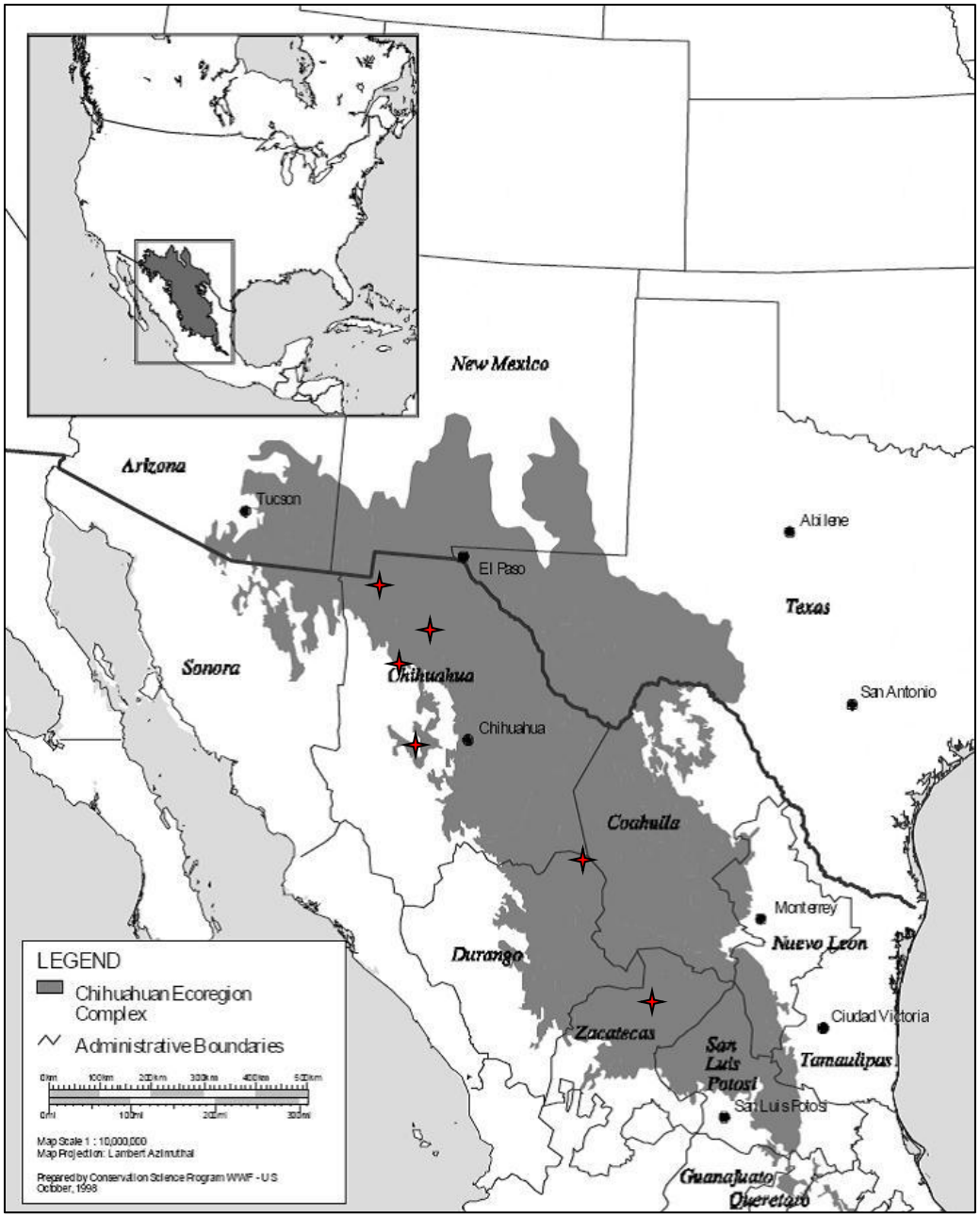


Figure 4.1. Location of the Chihuahuan Desert Ecoregion with the six wetlands selected for a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. Modified from Dinerstein et al. (2001). (Used with permission: Conservation Science Program WWF-US, 1998).

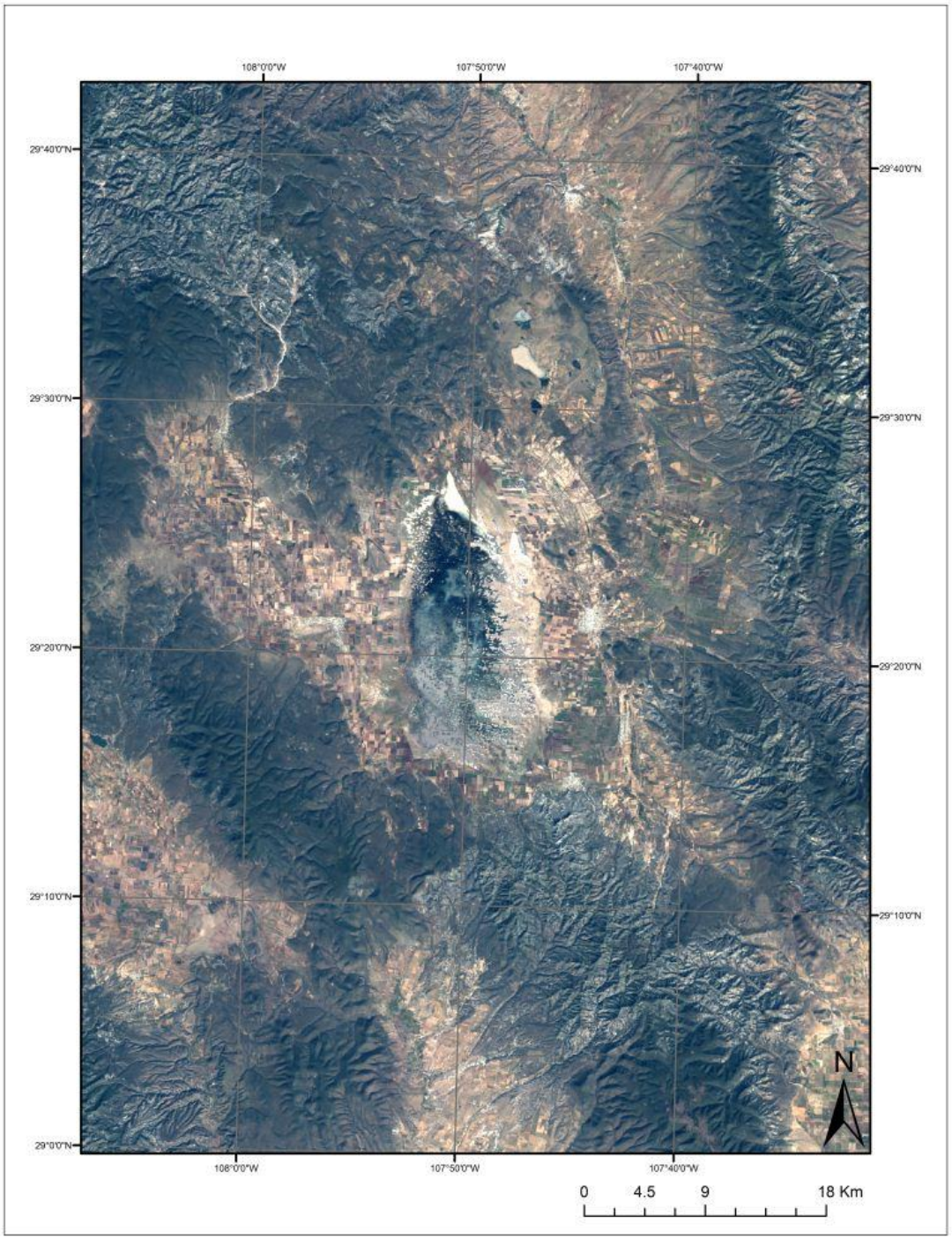


Figure 4.2. Satellite image of the study area located in the Laguna de Babícora, Chihuahua, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is surrounded by abundant food resources.

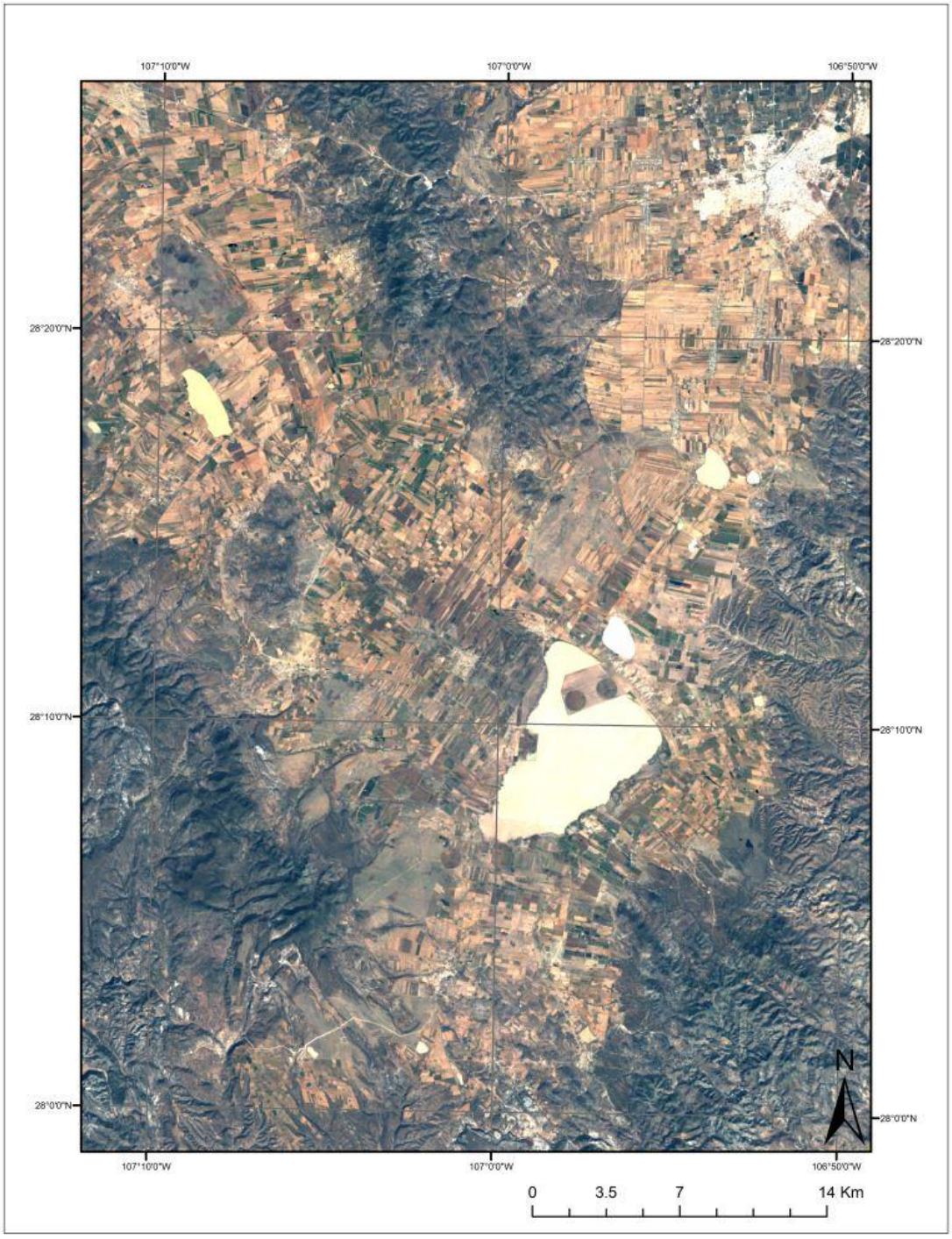


Figure 4.3. Satellite image of the study area located in the Laguna de Mexicanos, Chihuahua, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is surrounded by abundant food resources.

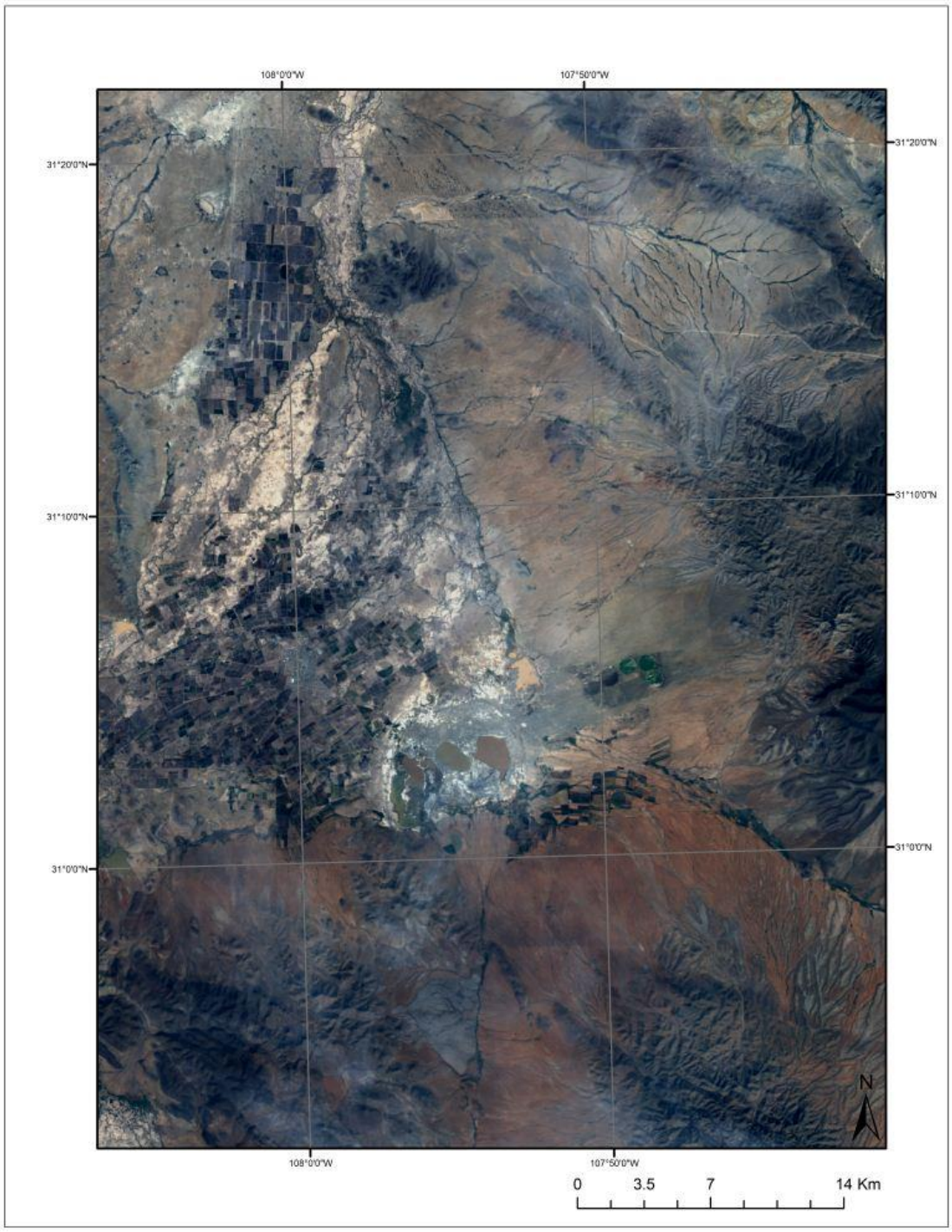


Figure 4.4. Satellite image of the study area located in the Laguna de Ojo Federico, Chihuahua, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is situated relatively close to food resources.

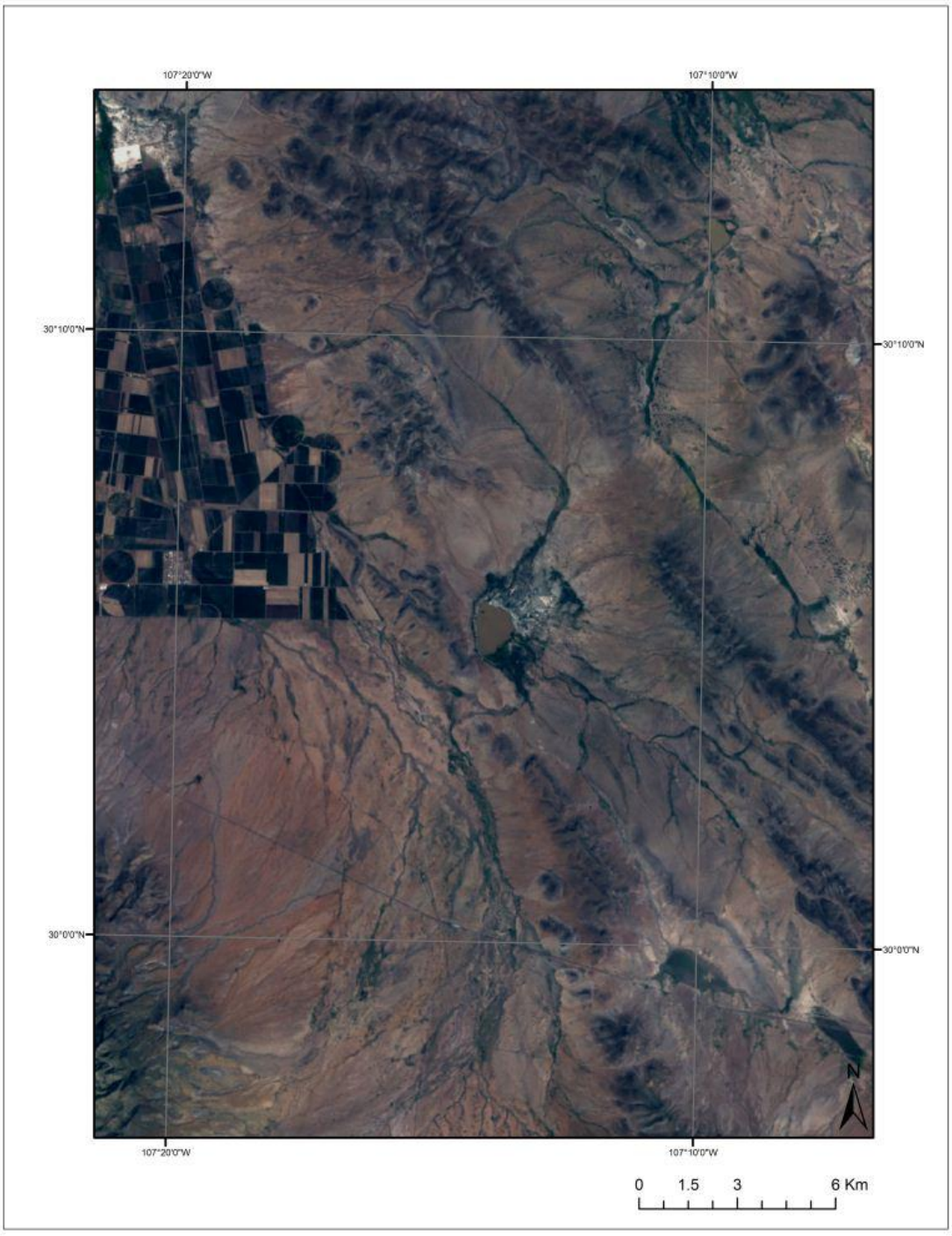


Figure 4.5. Satellite image of the study area located in the Laguna Victorio, Chihuahua, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is situated relatively close to food resources.

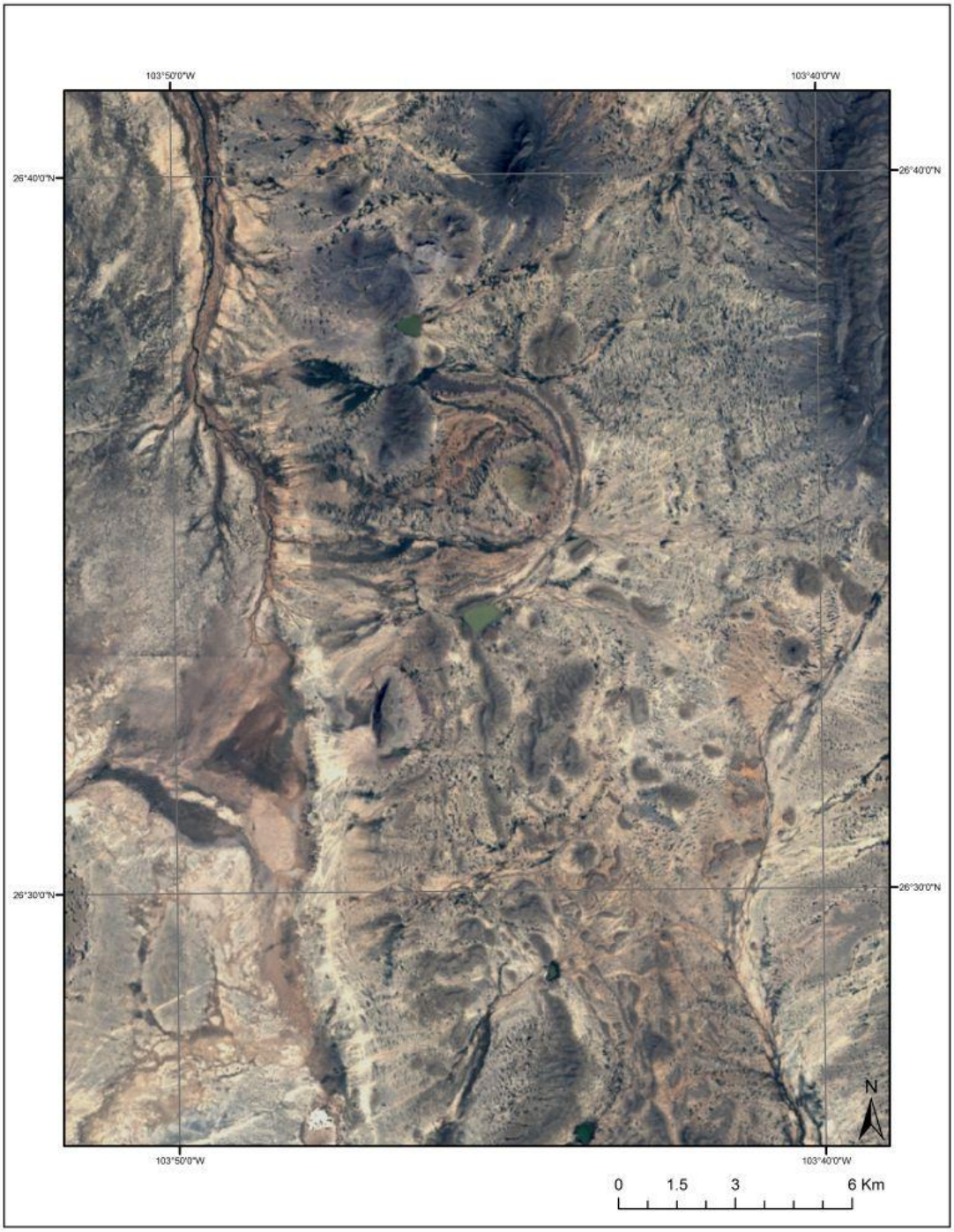


Figure 4.6. Satellite image of the study area located in the Presa San Carlos de Mapimí, Durango, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is situated far from food resources.


Figure 4.7. Satellite image of the study area located in the Laguna de San Juan de Ahorcados, Zacatecas, Mexico included in a diet study of Sandhill Cranes (*Grus canadensis*) during the winter (October to February) of 2007/08 and 2008/09. This wetland is situated far from food resources.

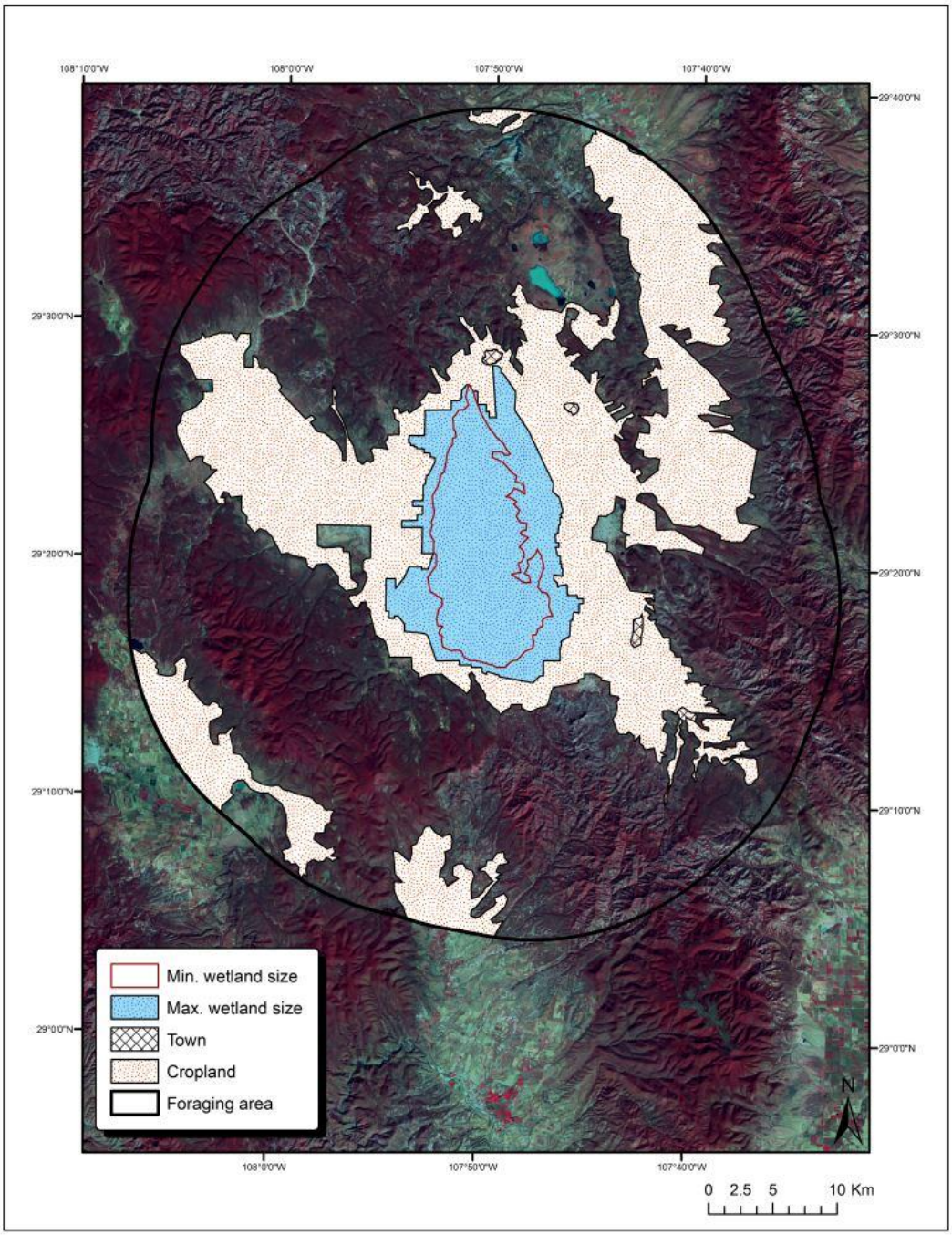


Figure 4.8. Satellite image of the Laguna de Babícora, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as high in crop availability.

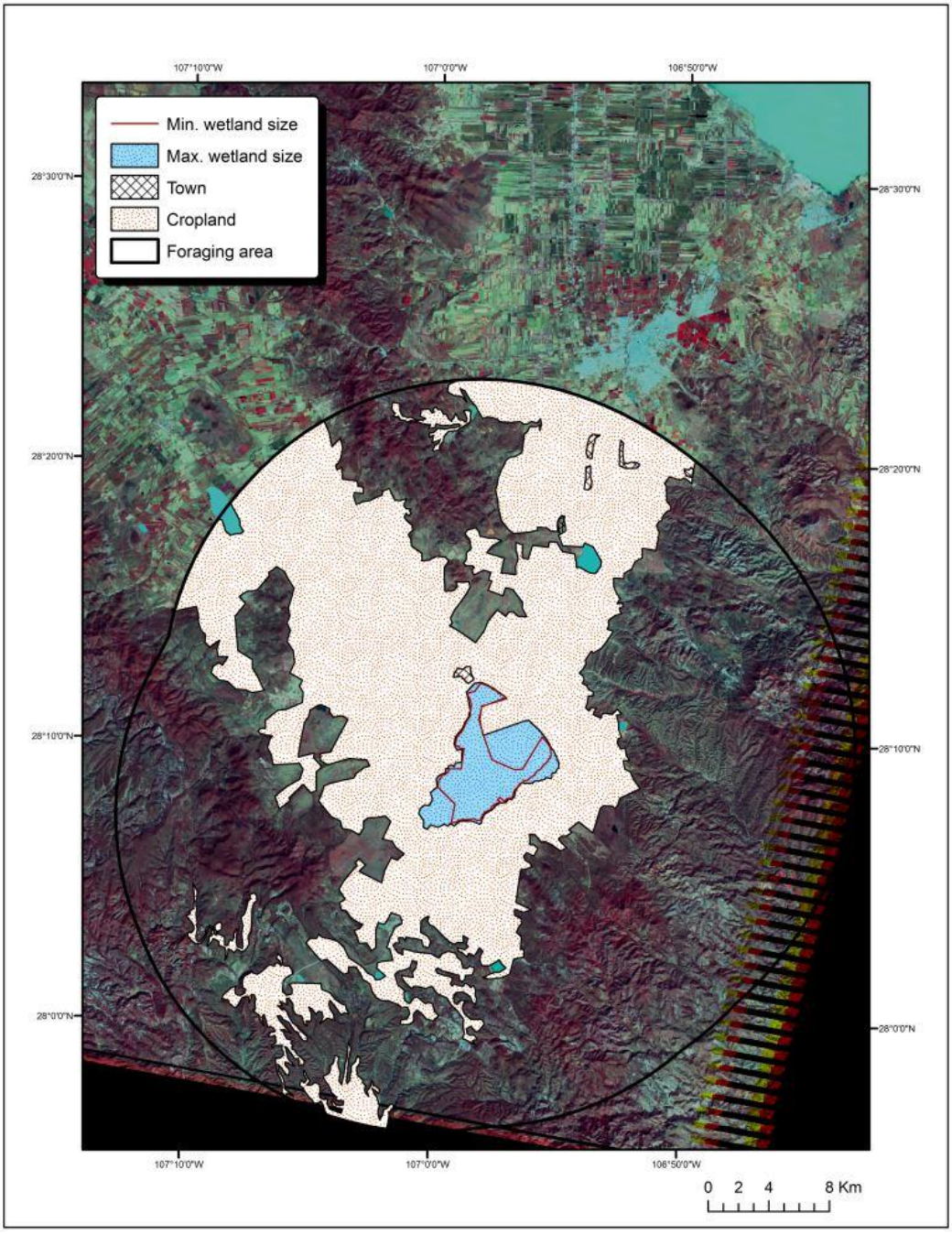


Figure 4.9. Satellite image of the Laguna de Mexicanos, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as high in crop availability.

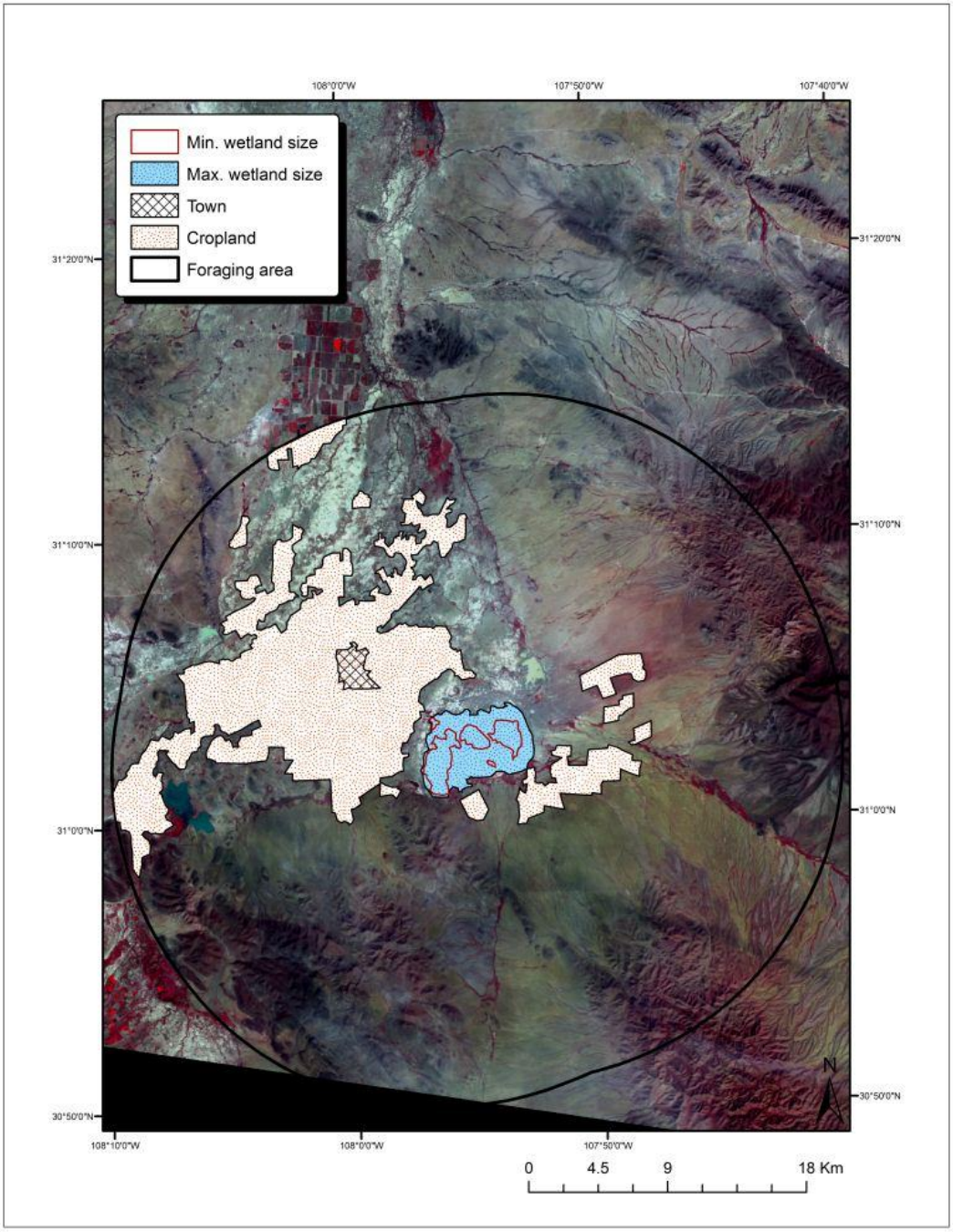


Figure 4.10. Satellite image of the Laguna de Ojo Federico, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as medium in crop availability.

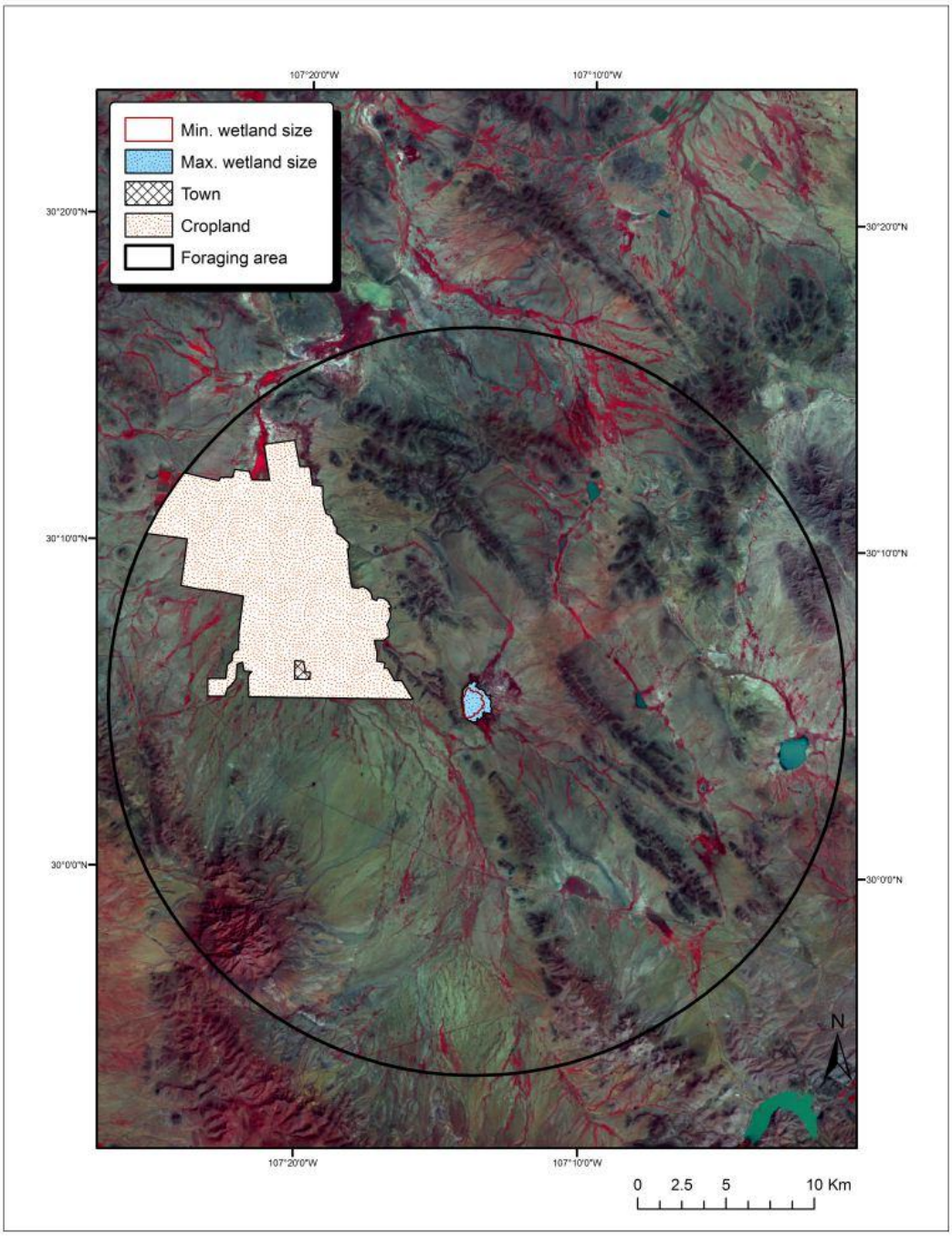


Figure 4.11. Satellite image of the Laguna Victorio, Chihuahua, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as medium in crop availability.

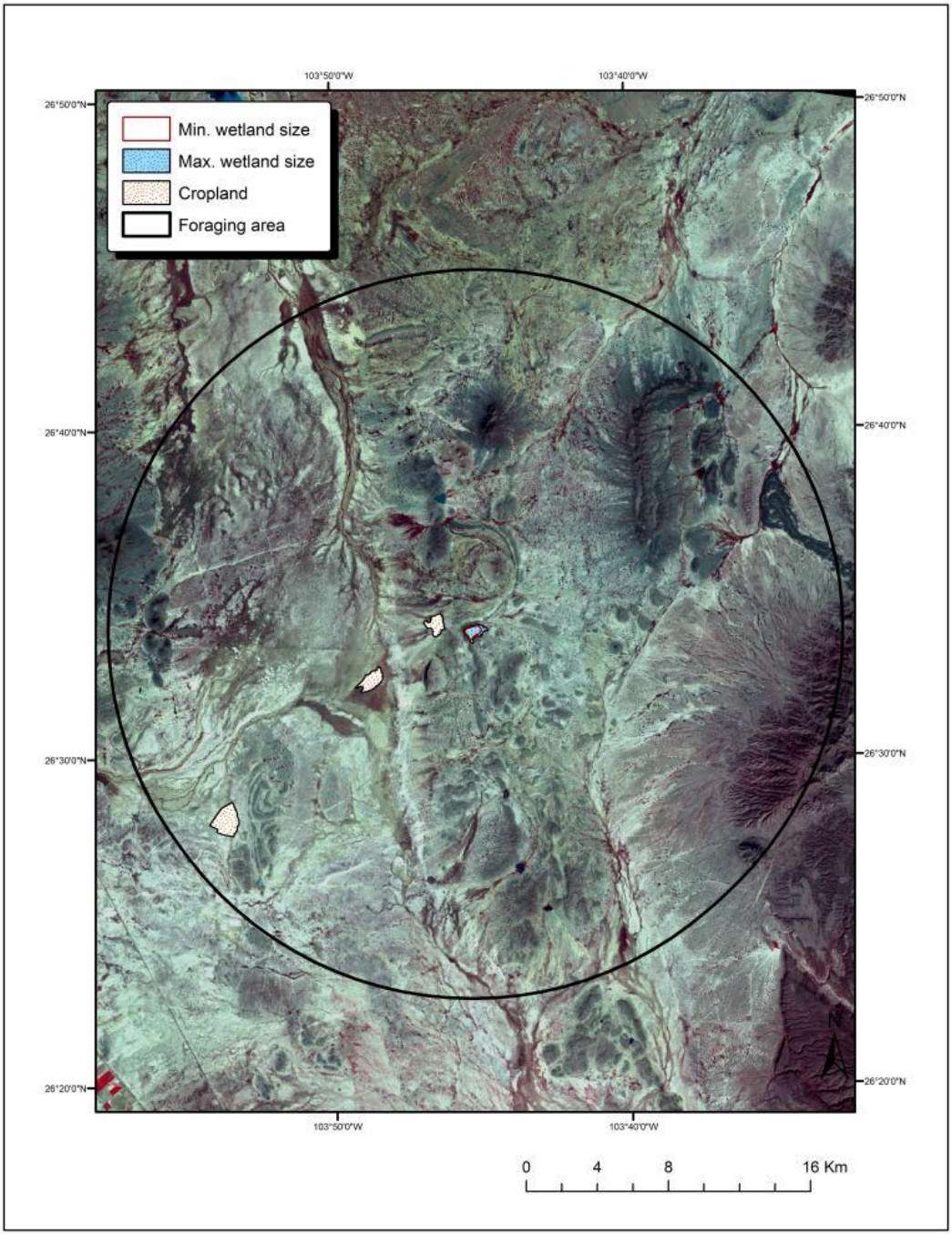


Figure 4.12. Satellite image of the Presa San Carlos de Mapimí, Durango, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as low in crop availability.

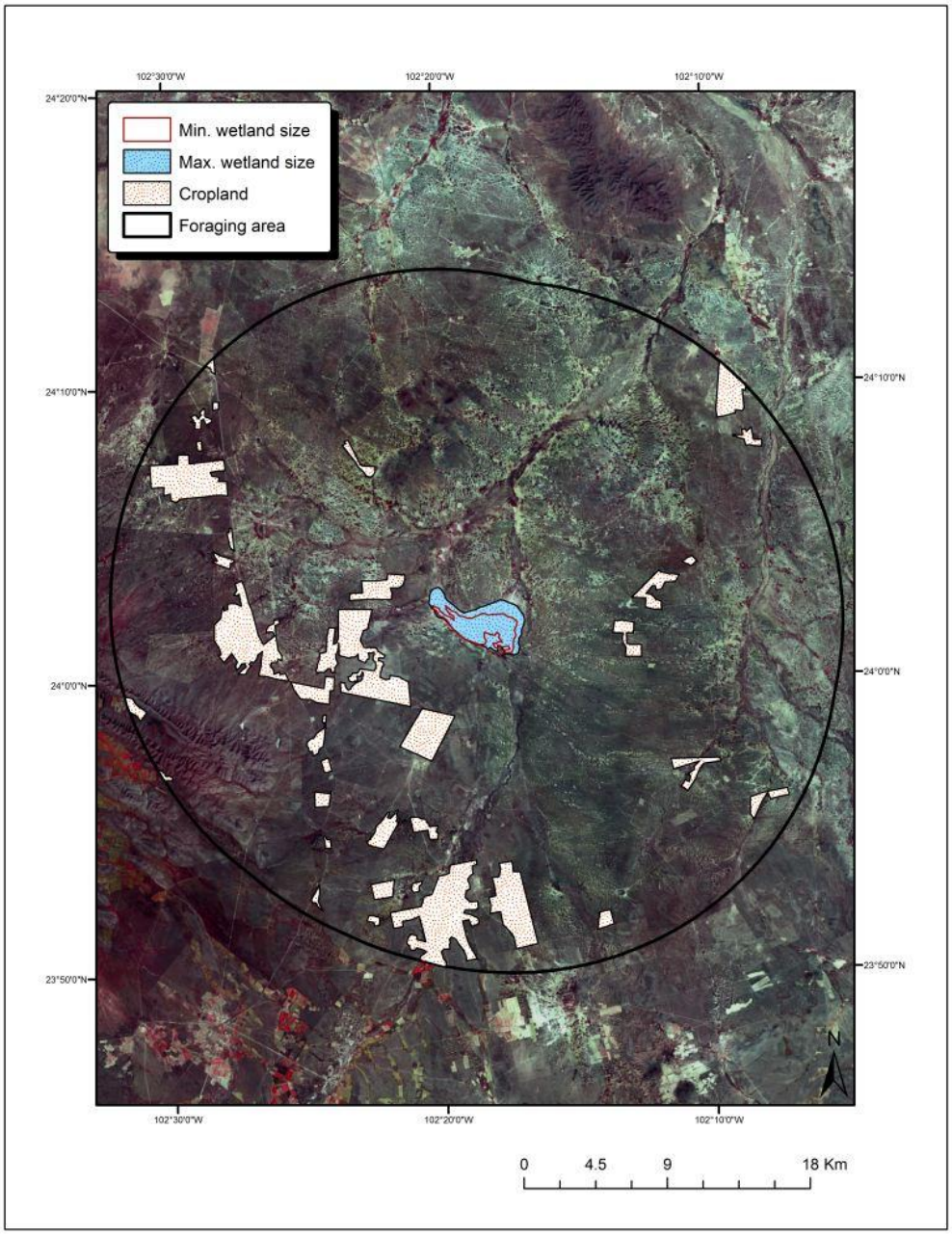


Figure 4.13. Satellite image of the Laguna de San Juan de Ahorcados, Zacatecas, indicating the wetland extent and the location and land cover of agriculture within a 20 km buffer zone (crane foraging area) around the wetland included in a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. This wetland was classified as low in crop availability.

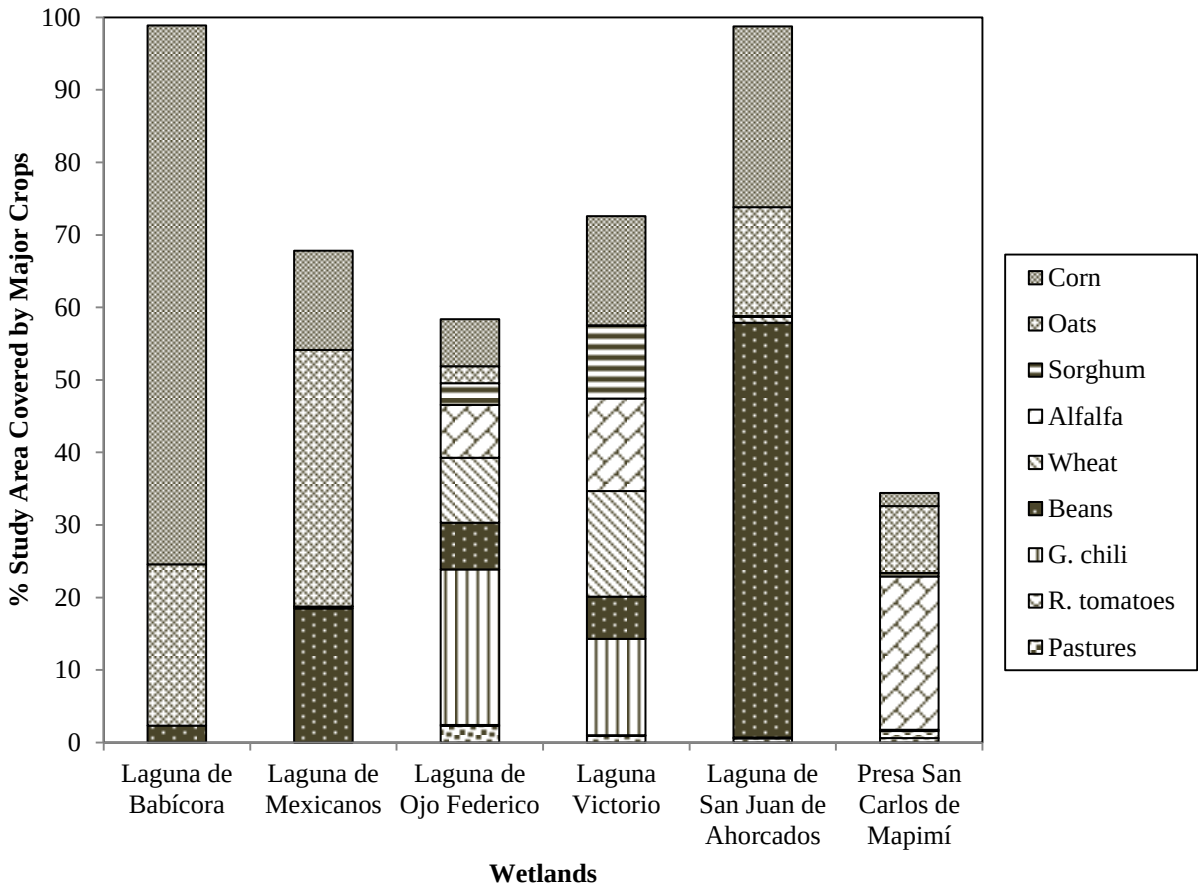


Figure 4.14. Percentage of major crops sown in the municipalities where the wetlands for a diet study of Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 were located. Municipalities containing the wetlands were Gómez Farías, Cusihuiriachi, Ascención, Buenaventura, General Francisco R. Murguía, and Tlahualilo. Data reported by the Sistema Estatal y Municipal de Bases de Datos (SIMBAD 2012) and obtained from the Instituto Nacional de Estadística y Geografía (INEGI 2012). The percentage reflects the mean value of the three years while the study was conducted (2007, 2008, and 2009).

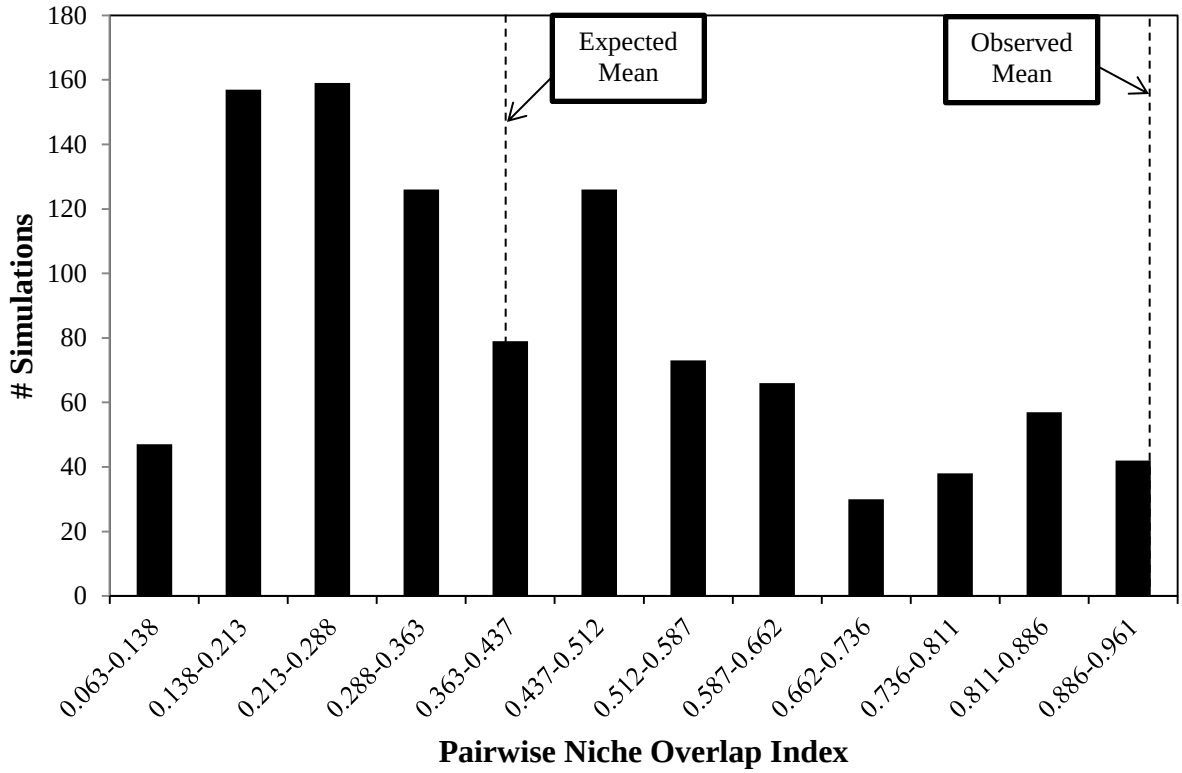


Figure 4.15. Histogram of simulation frequencies using Pianka's index to estimate diet overlap of Sandhill Cranes (*Grus canadensis*) in northern Mexico between the seasons (October to February) of 2007/08 and 2008/09. Observed $\bar{x}$ = 0.961, estimated $\bar{x}$ = 0.422, $\sigma^2$ = 0.052. (The estimated mean corresponds to the mean of 1,000 simulated iterations followed by its variance). The probability of obtaining an observed mean bigger or equal to the expected mean was 0.001.

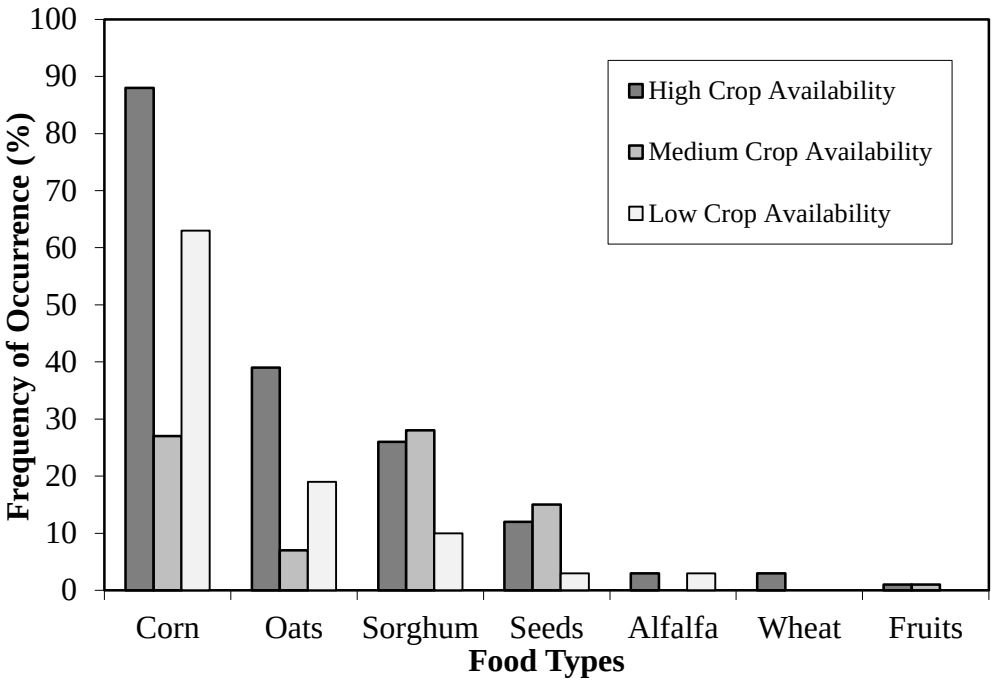


Figure 4.16. Frequency of occurrence of food types found in fecal samples of Sandhill Cranes (*Grus canadensis*) collected from six wetlands with high (*n* = 151), medium (*n* = 77), and low (*n* = 92) food availability for a diet study in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

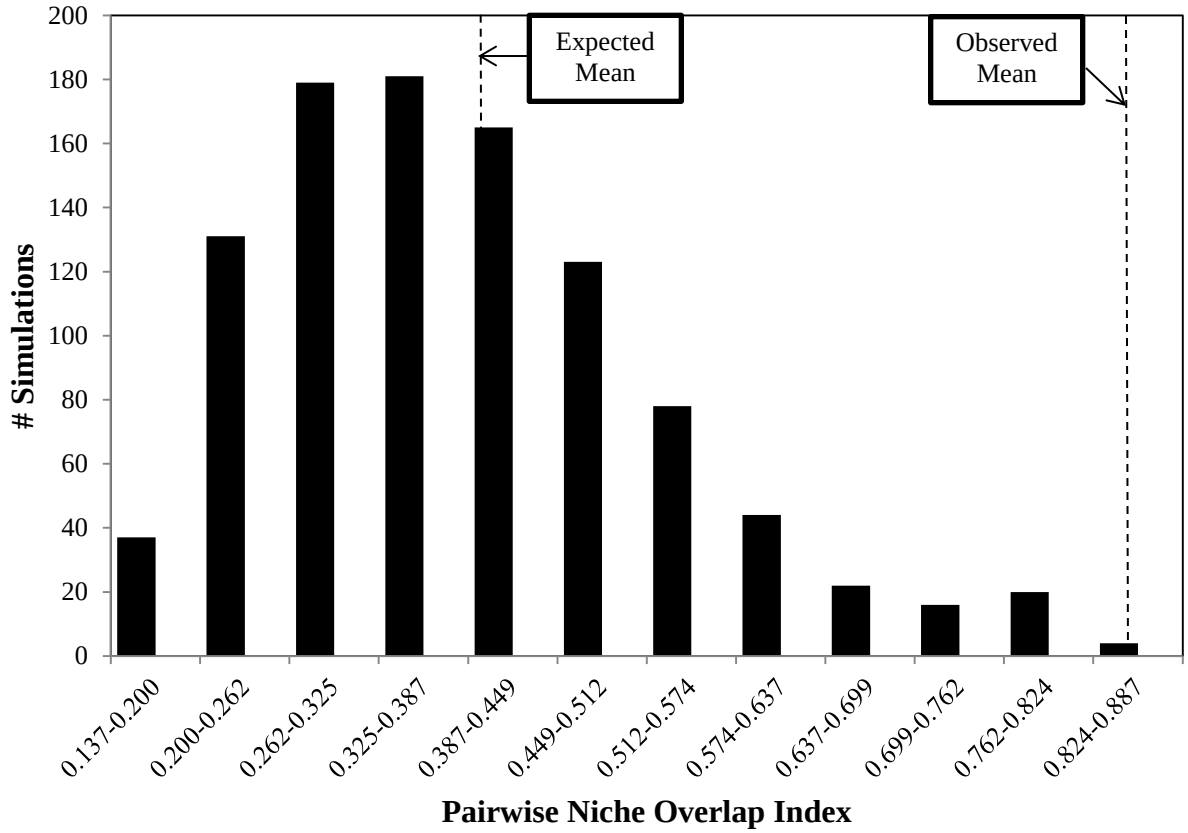


Figure 4.17. Histogram of simulation frequencies using Pianka's index to estimate diet overlap of Sandhill Cranes (*Grus canadensis*) between wetlands included in a diet study in northern Mexico during the winter (October to February) of 2007/08 and 2008/09. Observed $\bar{x}$ = 0.862, estimated $\bar{x}$ = 0.396, $\sigma^2$ = 0.020. (The estimated mean corresponds to the mean of 1,000 simulated iterations followed by its variance). The probability of obtaining an observed mean bigger or equal to the expected mean was 0.002.

# CHAPTER 5: RURAL INHABITANT PERCEPTIONS OF SANDHILL CRANES IN WINTERING AREAS OF NORTHERN MEXICO

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## ABSTRACT

Trends in the mid-continent population of Sandhill Cranes (*Grus canadensis*) indicate that the species is increasing. A large proportion of this population winters in northern Mexico where little information is available regarding human perceptions and possible conflicts between local inhabitants and cranes can occur. We conducted interviews of 40 rural inhabitants living near wetlands used by cranes in three Mexican states. All interviewees had knowledge of cranes and were capable of describing them. The arrival of cranes affected 43% of interviewees. The negative effects were mainly destroyed crops with a subsequent diminished production. Seventy percent of those affected implemented scare tactics to deter the birds, while others (15%) did nothing to mitigate crop losses and accepted such damages. While Sandhill Cranes continue to increase, conflicts with humans are expected to rise. Our results provide information about human attitudes towards cranes and can serve as the basis for future conservation guidelines.

## INTRODUCTION

Birds represent a major source of human-wildlife conflict and cause of agriculture damage worldwide (Weatherhead et al. 1982, Messmer 2000, Marzluff et al. 2001). Forms of agricultural damage include destruction and depredation of crops often caused by common species (Conover and Decker 1991, Dolbeer 1998, Reiter et al. 1999). In North America, substantial losses in agricultural productivity have been quantified for species such as Canada geese (*Branta canadensis*), snow geese (*Chen caerulescens*) (Conover et al. 1995, Ankney 1996, Aubry et al. 2010), red-winged blackbirds (*Agelaius phoeniceus*) (Dolbeer 1990), and European starlings (*Sturnus vulgaris*) (White et al. 1985). Sandhill Cranes (*Grus canadensis*) represent another common North American species that may potentially affect agriculture as they are increasing in abundance, expanding in range (Krapu et al. 2011), and consuming mainly human crops (e.g., corn) (Iverson et al. 1982, Krapu et al. 1984, Reinecke and Krapu 1986).

An estimated 14% (70,000) of the mid-continent population of Sandhill Cranes winters in northern Mexico (Drewien et al. 1996, Krapu et al. 2011). However few details exist on their diet and foraging behavior in the region. Wintering areas occur mainly in the Chihuahuan Desert in north-central Mexico, where farmers grow their crops in arid and semiarid environments (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad [CONABIO], 1998). Guzman-Aranda (1995) suggested that crop damage occurred by Sandhill Cranes and represented a major problem in some areas such as Laguna de Babícora, Chihuahua. While human tolerance to crop losses in other countries may be motivated by financial compensation programs (Wagner et al. 1997), this is not the case in Mexico where such programs do not exist and farming operations

are small and struggle to compete with highly subsidized corn producers in the USA and Canada (Koechlin and Larudee 1992).

Despite the large number of cranes wintering in Mexico, little information is available regarding crane ecology and interactions with humans. Cranes occupy wetlands in northern Mexico, which receive official protection by the federal government (Diario Oficial de la Federación [DOF], 1992). However, little implementation and enforcement of environmental legislation occurs (Szekely et al. 2005). Most wetlands are classified as Modified Rural Landscapes (MDRLs), that is, major landscapes found outside natural protected areas, with high biodiversity, that often suffer from highly destructive management regimes (Little 1994). Successful biodiversity conservation in these wetlands requires a collaborative agreement among interested parties, particularly among those stakeholders facing crop damage (Grimble and Wellard 1997). As the population of Sandhill Cranes continues to increase and expand their wintering range in northern Mexico (Lopez-Saut et al. 2011), conflicts between humans and cranes are expected to increase. Effective management decisions require information regarding the perceptions that people have in the areas where cranes winter (Knuth et al. 1992). This article contributes to that knowledge and represents the first account of human attitudes towards wildlife damage in northern Mexico.

The article describes the social context of wintering habitat for Sandhill Cranes in northern Mexico. Our objectives were to: (a) document the perceptions that local inhabitants have toward Sandhill Cranes; (b) determine the proportion of farmers who considered that cranes affected their livelihood; (c) document methods used to mitigate

those effects; and (d) assess such methods and provide recommendations for management.

## MATERIALS AND METHODS

### *Study Area*

The study area included wetlands distributed within the wintering range of Sandhill Cranes in northern Mexico. This area comprises the Chihuahuan Desert Ecoregion as described by WWF (Dinerstein et al. 2001) (Figure 5.1). This region stretches north-south between central southern USA and into central Mexico, where it includes a big portion of the state of Chihuahua, most of Coahuila, east of Durango, north of Zacatecas, north and central San Luis Potosi, and some small areas of Nuevo Leon and Tamaulipas.

Wetlands represent critical habitat for cranes providing roosting sites during the night and resting sites during the hottest hours of the day. We concentrated our efforts around four wetlands where cranes had been recorded historically. The selected wetlands included (a) Laguna de Mexicanos (Chihuahua); (b) Presa San Carlos (Reserva de la Biosfera del Bolsón de Mapimí in Durango); (c) Laguna de Santiaguillo (Durango); and (d) Laguna de San Juan de Ahorcados (Zacatecas) (Table 5.1).

### *Data Collection*

During a study of winter ecology of Sandhill Cranes in northern Mexico in 2007-2008 we included a preliminary human dimensions survey. Following the findings for Laguna de Babícora, Chihuahua (Guzman-Aranda 1995) we designed interviews to characterize rural inhabitants' perceptions on Sandhill Cranes and crop damage. The

survey included 15 questions in Spanish, nine open-ended unstructured and six dichotomous structured questions (Table 5.2). The answers were transcribed for analysis. For each participant, sex, town, and profession was observed and annotated. Since the study concentrated on inhabitants whose profession was farming, we interviewed 40 farmers living around four wetlands where cranes roosted (10 people per wetland). In each of our four study sites, we approached residents about participating in our study, interviewed the first person who agreed, and employed a snowball sampling method thereafter. Snowball sampling uses information provided by insiders to locate people willing to participate in the study (Biernacki and Waldorf 1981, Lopes et al. 1996). It is useful when the focus of interest may involve an illegal behavior because it provides access to hidden participants that would otherwise be difficult for the interviewer to locate. Our interviews included sensitive questions regarding shooting of cranes, an illegal activity in Mexico when done outside areas designated for hunting. This resulted in participants admitting their reservations to collaborate or answer honestly by being afraid of potential legal consequences. In those cases, participants would direct us to other neighbors or family members who would be willing to participate without reservations.

Some of the wetlands included in the study were located in extremely isolated areas and inhabited only by a few families. In these cases our sample size included the population of possible interviewees. We believe that the study represents the perceptions of people living in close proximity to cranes.

**RESULTS**

Most interviewees (90%) were males between 20 and 80 years old. These findings reflect a socio-demographic scenario in northern rural Mexico where most farmers are males (Stoleson et al. 2005). All interviewees indicated that they had knowledge of cranes, saw them regularly, and were capable of describing them. Winter (December, January, and February) was the season most respondents (78%) reported seeing cranes followed by fall (September, October, and November) (20% of respondents). The three most common areas for crane observations were shallow areas of lakes (56%), agriculture fields (35%), and cattle rangelands (2%). Two-fifths (41%) of participants had seen up to100 cranes at a time, while larger flocks were reported less frequently, 500 to 1,000 cranes (10%), and ≥ 5,000 cranes (2%). Interviewees reported cranes eating corn (66%), oats (21%), sorghum (5%), and other items including wheat, insects, and cow droppings (2% each). Foraging was observed in agriculture fields (83%) and lakes (15%). The majority of respondents did not know where cranes came from (71%), while smaller percentages mentioned Canada (24%) and USA (2%). Nine out of 10 said they did not hunt cranes, 5% mentioned they used to hunt them, and 5% said they still hunt them.

Forty-three percent of participants believed that cranes caused harm to their crops. Of those farmers who declared crop damage, 52% responded that cranes consumed harvested corn that was left packed and drying in the fields, 38% responded that cranes consumed unharvested mature corn that was left in the fields, and 7% responded that cranes consumed newly planted corn that was just starting to grow.

Finally, regarding the methods used to reduce crop damage, 70% of the participants responded that they used scare tactics to deter the birds (e.g., making noise, driving close to cranes, and use of scarecrows), 15% did nothing and accepted such loses, 5% adjusted their harvesting times in order to avoid the time that cranes were using the fields, 5% increased vigilance of the crops while the cranes were in the area, and 5% shot the cranes if they were feeding on their fields.

**DISCUSSION**

Cranes feed on most stages of corn (Iverson et al. 1982, Krapu et al. 1984, Barzen and Lacy 2011). Avoiding harvest times that coincide with crane arrival as some respondents reported doing, could decrease crop damage and conflict between cranes and humans. On the other hand, respondents who admitted to harvesting and leaving the corn in the fields to dry, considered cranes to affect their yield and admitted using scare tactics. Such practices can lead to daily disturbances during a critical life history time for cranes potentially deterring them from a roosting or feeding site.

Our study was similar to Guzman-Aranda's (1995) which investigated human attitudes towards Sandhill Cranes in Mexico. However, his study focused only on one site, Laguna de Babícora, Chihuahua, while we extended the survey area to include a broader range of wetlands. According to Guzman-Aranda (1995) crop damage in this wetland had become a major problem, where 50% of interviewed landowners suffered crop losses due primarily to Sandhill Cranes followed by geese (*Anser* spp.). Although the results of both studies were similar, it must be noted that comparison between human dimension studies should be done carefully because attitudes and perceptions of wildlife are different between countries and the magnitude of the problem varies by region

(McIvor and Conover 1994). For instance, most corn producers in the USA are likely to tolerate some crop losses due to wildlife and most will absorb losses of <1% of their crops (McIvor 1993, Wywialowski 1996). This may not be the case in our study area where farming operations are much smaller and more vulnerable to weather conditions because of the lack of irrigation. On the other hand, farmer's tolerance to wildlife damage in the USA and Canada may be driven by financial compensation (Wagner et al. 1997).

The number of respondents who admitted killing cranes as a method to reduce crop damage was much lower than in McIvor & Conover (1994), the only study that has been done regarding human attitudes towards cranes in the USA. Their study investigated the different perceptions between farmers and non-farmers towards the Rocky Mountain population of Sandhill Cranes in Utah and Wyoming. Forty-nine percent of farmers ranked Sandhill Cranes as frequent and severe consumers of their crops and 69% preferred to hunt them as a control method to reduce crop damage. Regulated hunting in Mexico is mainly practiced by sport hunters coming from the USA (Stoleson et al. 2005). Fewer Mexicans have access to hunting equipment and hunting is only allowed in a few designated areas. This may explain why hunting of cranes as a control method was less of an option in our study area.

Wildlife populations that increase in size due to protection measures, change in management approaches, and increased crop acreages are prone to cause conflicts with rural residents (Conover and Decker 1991, Messmer 2000). As the mid-continent population continues to increase (Sharp and Vogel 1991) and more land is converted to human uses, so will the number of cranes migrating into northern Mexico that is already

estimated in 70,000 individuals (Drewien et al. 1996). Recent studies indicate that cranes are increasing and expanding their wintering range in northern Mexico (Lopez-Saut et al. 2011). Our data suggests that cranes are causing conflicts with farmers and are viewed as a problem in this region of their wintering range. In wetlands where cranes congregate in large numbers, the impacts on field crops can be substantial.

Our results offer a glimpse of the attitudes of rural inhabitants towards cranes. The fact that most people could identify cranes in rural areas represents the first step toward possible implementation of conservation efforts in wintering sites. Although the majority of respondents had a positive attitude towards cranes and did not consider they inflicted crop damage, the amount of affected farmers was substantial. Furthermore, this result could easily change in the near future as the Sandhill Crane population continues to increase and conflicts between humans and cranes can potentially rise in the next decade. Conservation organizations and state agencies in Mexico and USA should expect an increase in crane-human interactions and be prepared to implement management strategies and educational programs to mitigate such impacts. Perhaps a starting point as our data suggest could be for NGOs and agencies to encourage farmers to harvest the fields before the arrival of the cranes. This could be possible during years when the rainy season is on time allowing farmers to plant earlier. When harvesting is late due to weather conditions, however, the use of nontoxic avian foraging repellents may be a solution. Chemical seed treatments are being tested as deterrent methods to protect crops in areas where cranes have become a problem (Blackwell et al. 2001, Barzen and Lacy 2011). Nonetheless, an economic analysis of the costs of crop damage and potential

strategies to avoid conflict would be helpful, now that the baseline information is available.

## ACKNOWLEDGEMENTS

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## LITERATURE CITED

Ankney, C. D. 1996. An embarrassment of riches: too many geese. Journal of Wildlife Management 60:217-223.

Aubry, L. M., R. F. Rockwell, and D. N. Koons. 2010. Metapopulation dynamics of mid-continent lesser snow geese: implications for management Human Wildlife Interactions 4:170-191.

Barzen, J., and A. Lacy. 2011. A sustainable solution for crop damage by cranes and other bird species to planted seed. Joint Meeting of 11th North Amercian Crane Workshop and 34th Annual Meeting of The Waterbird Society, Grand Island, NE.

Biernacki, P., and D. Waldorf. 1981. Snowball sampling: Problems and techniques of chain referral sampling. Sociological Methods & Research 10:141-163.

Blackwell, B. F., D. A. Helon, and R. A. Dolbeer. 2001. Repelling sandhill cranes from corn: whole-kernel experiments with captive birds. Crop Protection 20:65-68.

Burleson, D. R. 1980. Elementary statistics. Winthrop Publishers, Cambridge, MA.

Castelli, P. M., and S. E. Sleggs. 2000. Efficiency of border collies to control nuisance Canada geese. Wildlife Society Bulletin 28:385-392.

CONABIO. 1998. La diversidad biologica de Mexico: Estudio de Pais, 1998. Comision Nacional para el Conocimiento y Uso de la Biodiversidad. Mexico.

Conover, M. R., and D. J. Decker. 1991. Wildlife damage to crops: perceptions of agricultural and wildlife professionals in 1957 and 1987. Wildlife Society Bulletin 19:46-52.

Conover, M. R., W. C. Pitt, K. K. Kessler, T. J. DuBow, and W. A. Sanborn. 1995. Review of human injuries, illnesses, and economic losses caused by wildlife in the United States. Wildlife Society Bulletin 23:407-414.

Dinerstein, E., D. Olson, J. Atchley, C. Loucks, S. Contreras-Balderas, R. Abell, E. Iñigo, E. Enkerlin, C. Williams, and G. Castilleja. 2001. Ecoregion-Based Conservation in the Chihuahuan Desert: A Biological Assessment. WWF, CONABIO, TNC, PRONATURA Noreste, and ITESM.

DOF. 1992. Ley de Aguas Nacionales. Diario Oficial de la Federacion.

Drewien, R. C., W. M. Brown, and D. S. Benning. 1996. Distribution and abundace of sandhill cranes in Mexico. Journal of Wildlife Management 60:270-285.

Gipson, P. S., and W. B. Ballard. 1998. Accounts of famous North American wolves. The Canadian Field-Naturalist 112:724-739.

Grimble, R., and K. Wellard. 1997. Stakeholder methodologies in natural resource management: A review of principles, contexts, experiences and opportunities. Agricultural Systems 55:173-193.

Guzman-Aranda, J. C. 1995. Landowner wildlife conservation attitudes at Laguna de Babicora, Chihuahua, Mexico New Mexico State University, Las Cruces, NM.

Iriarte, J. A., W. L. Franklin, W. E. Johnson, and K. H. Redford. 1990. Biogeographic variation of food habits and body size of the America puma. Oecologia 85:185-190.

Knuth, B. A., R. J. Stout, W. F. Siemer, D. J. Decker, and R. C. Stedman. 1992. Risk management concepts for improving wildlife population decisions and public communication strategies. Transactions of the North American Wildlife and Natural Resources Conference 57:63-74.

Krapu, G. L., D. A. Brandt, K. L. Jones, and D. H. Johnson. 2011. Geographic distribution of the Mid-Continent Population of sandhill cranes and related management applications Wildlife Monographs 175:1-38.

Little, P. D. 1994. The link between local participation and improved conservation: A review of issues and experiences. Pages 347-372 *in* D. Western, S. C. Strum, and R. M. Wright, editors. Natural connections: Perspectives in community-based conservation. Island Press, Washington, DC.

Lopes, C. S., L. C. Rodrigues, and R. Sichieri. 1996. The lack of selection bias in a snowball sampled case-control study on drug abuse. International Journal of Epidemiology 25:1267-1270.

Lopez-Saut, E. G., F. Chavez-Ramirez, and R. Rodriguez-Estrella. 2011. New records of wintering grounds for sandhill cranes in Mexico. Waterbirds 34:239-246.

McBride, R. T. 1976. The status and ecology of the mountain lion (*Felis concolor stanleyana*) in the Texas-Mexico border. Sul Ross State University, Alpine, Texas, USA.

McIvor, D. E. 1993. Incidence and perceptions of sandhill crane crop depredations. Utah State University, Logan.

McIvor, D. E., and M. R. Conover. 1994. Perceptions of farmers and non-farmers toward management of problem wildlife. Wildlife Society Bulletin 22:212-219.

Messmer, T. A. 2000. The emergence of human-wildlife conflict management: turning challenges into opportunities. International Biodeterioration & Biodegradation 45:97-102.

Reed, J. E., W. B. Ballard, P. S. Gipson, B. T. Kelly, P. R. Krausman, M. C. Wallace, and D. B. Wester. 2006. Diets of free-ranging Mexican gray wolves in Arizona and New Mexico. Wildlife Society Bulletin 34:1127-1133.

Rosas-Rosas, O. C., R. Valdez, L. C. Bender, and D. Daniel. 2003. Food habits of pumas in Northwestern Sonora, Mexico. Wildlife Society Bulletin 31:528-535.

SEMARNAT. 2010. Proteccion ambiental - Especies nativas de Mexico de flora y fauna silvestres - Categorias de riesgo y especificaciones para su inclusion, exclusion o cambio - Lista de especies en riesgo. Diario Oficial de la Federacion NOM-059-SEMARNAT-2010.

Sharp, D. E., and W. O. Vogel. 1991. Population status, hunting regulations, hunting activity, and harvest of mid-continent sandhill cranes. Proceeding of the North American Crane Workshop 6:24-32.

Szekely, A., L. O. Martinez Morales, M. J. Spalding, and D. Cartron. 2005. Mexico's legal and institutional framework for the conservation of biodiversity and ecosystems. Page 496 *in* J. E. Cartron, G. Ceballos, and R. Stephen Felger, editors. Biodiversity, ecosystems, and conservation in Northern Mexico. Oxford University Press, Inc., New York, NY.

Wagner, K. K., R. H. Schmidt, and M. R. Conover. 1997. Compensation programs for wildlife damage in North America. Wildlife Society Bulletin 25:312-319.

Wywialowski, A. P. 1996. Wildlife damage to field corn in 1993. Wildlife Society Bulletin 24:264-271.

Table 5.1. Location of the wetlands included in the survey on rural inhabitant attitudes towards Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter 2007-2008.

| Number | Wetland | Location | State | Towns |
|---|---|---|---|---|
| 1 | Laguna de Mexicanos | 28°10’36.44’’N 106°55’42.09’’W | Chihuahua | Ojo de Agua<br>Bajío de Abajo<br>Rancho González |
| 2 | Presa San Carlos | 26°34’2.11’’N 103°44’49.56’’W | Durango | La Flor<br>Rancho San Felipe |
| 3 | Laguna de Santiaguillo | 24°54’38.83’’N 104°52’27.28’’W | Durango | Castillo del Valle<br>Miguel Negrete<br>San Miguel de Allende |
| 4 | Laguna de San Juan de Ahorcados | 24°1’21.18’’N 102°17’48.14’’W | Zacatecas | San Juan de Ahorcados<br>San José de la Laguna |

Table 5.2. Summary and type of questions included in the survey on rural inhabitant attitudes towards Sandhill Cranes (*Grus canadensis*) in northern Mexico during the winter 2007-2008.

| Questions | Answers |
|---|---|
| 1. How old are you? | Open-ended unstructured |
| 2. Do you know what a crane is? | Dichotomous structured |
| 3. Can you distinguish them from herons and geese? | Dichotomous structured |
| 4. Can you describe a crane? | Open-ended unstructured |
| 5. Do you see cranes every year? | Dichotomous structured |
| 6. Which time of the year do you see cranes? | Open-ended unstructured |
| 7. In which areas do you observe cranes? | Open-ended unstructured |
| 8. How many individuals have you seen at a time? | Dichotomous structured |
| 9. Where do you see cranes foraging? | Open-ended unstructured |
| 10. What do you see cranes eating? | Open-ended unstructured |
| 11. Do you know from where cranes come from? | Open-ended unstructured |
| 12. Are you affected by the arrival of cranes? | Dichotomous structured |
| 13. How do cranes affect you? | Open-ended unstructured |
| 14. What methods do you use to mitigate such effects? | Open-ended unstructured |
| 15. Do you hunt cranes? | Dichotomous structured |

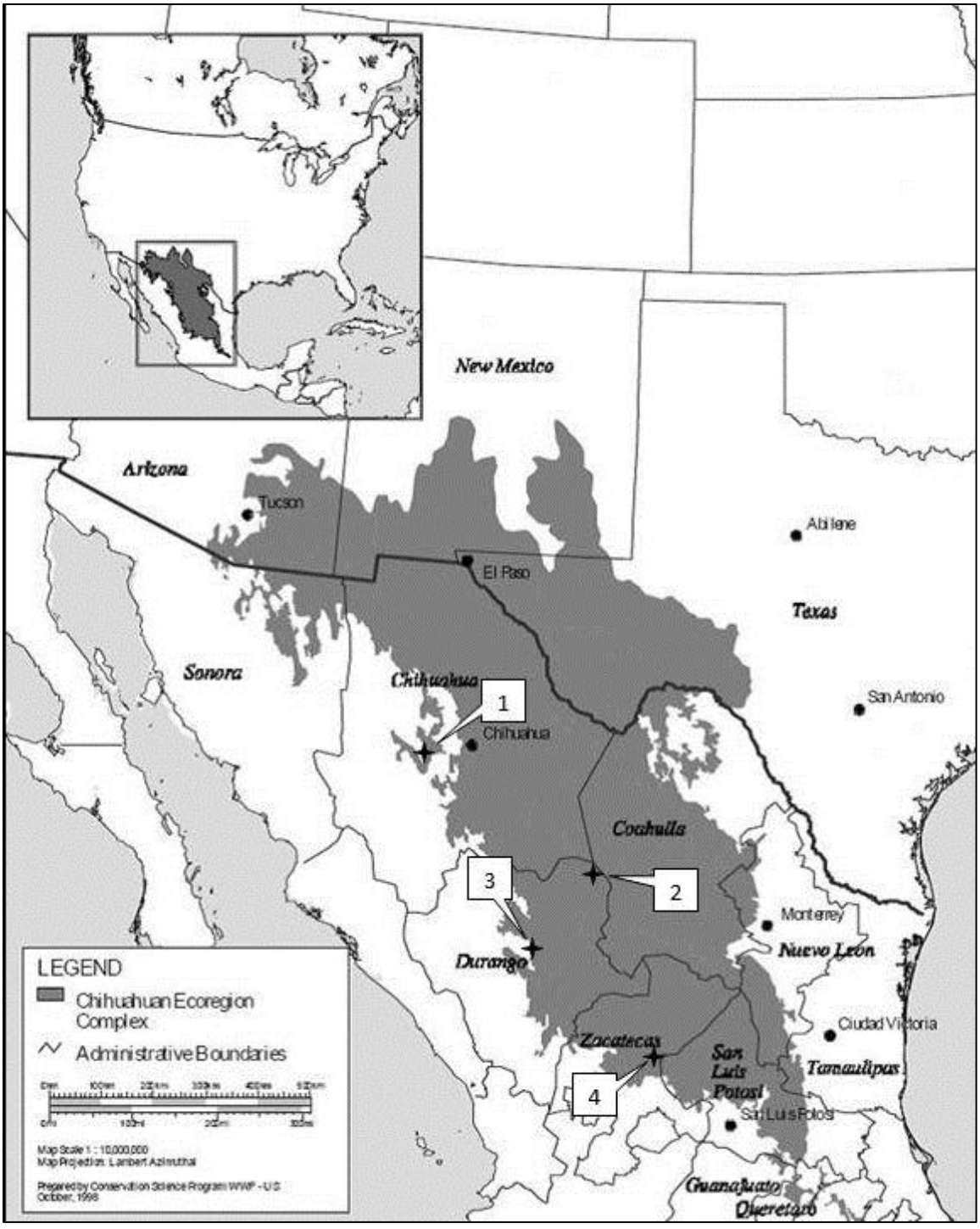


Figure 5.1. Location of the Chihuahuan Desert Ecoregion with the four wetlands selected for the study. Modified from Dinerstein et al. (2001). (Used with permission: Conservation Science Program WWF-US, 1998).

# CHAPTER 6: SUMMARY

Fourteen percent of the Mid-continent Population of Sandhill Cranes winters in northern Mexico, mainly in the states of Chihuahua, Durango, and Coahuila. The population is increasing in North America; however, the distribution of the species in Mexico has been reduced in the last four decades where Sandhill Cranes are considered a threatened species. Portions of the wintering grounds in northern Mexico have been extensively altered for agricultural development and many of the wetlands historically used by cranes have been degraded. The information provided by this dissertation fills up some of the gaps on winter ecology of Sandhill Cranes and supplies valuable results that can help predict future trends for the overall population.

This was the first study to measure the effects of environmental conditions on the physiological state of cranes in the wild. The physiological state of Sandhill Cranes varied significantly among sites where cranes were exposed to different environmental stress factors. The main environmental factor to have an effect on stress levels of Sandhill Cranes in Mexico was wetland size at their wintering sites. My data suggests that human induced changes to the landscape, such as excessive water extraction, can have measurable effects on cranes. Conservation of wetlands in northern Mexico is crucial for the continuity of the species in this arid region. Sandhill Cranes depend heavily on wetland habitat and are susceptible to water changes. Without wetlands of a substantial size, cranes would probably stop using this region as their wintering grounds. Future wetland conservation will be crucial to ensure that the proportion of cranes wintering in northern Mexico does not decrease.

Stress levels of Sandhill Cranes varied during the study period. My data suggests that stress levels are higher at the end of winter. This increase in corticosterone levels with time is probably due to a combination of factors including hotter and drier environmental conditions, increased competition due to fewer food resources, preparation for spring migration, and pair bonding in preparation for the breeding season.

I quantified the physiological state of Sandhill Cranes by measuring fecal glucocorticoid metabolites. Fecal glucocorticoid assays are a valuable method to assess stress hormones non-invasively in wild populations. This was the first study to validate the use of a commercially available corticosterone kit as a method for measuring glucocorticoids in feces of Sandhill Cranes. My data suggests that enzyme immunoassays provide accurate measurements of steroid metabolite concentrations comparable to traditional radioimmunoassays. Enzyme immunoassays offer safety and financial advantages, making it a reliable alternative for countries that may have limited access to laboratory instruments.

This was also the first study to quantify winter diet of Sandhill Cranes in Mexico. The results of my study confirmed that cranes wintering in Mexico fed mostly on agricultural grains coinciding with most previous studies. However, my results also suggest that cranes exhibit an advantageous specialized diet of corn since patterns of consumption did not vary with corn availability. Sandhill Cranes in northern Mexico did not behave as opportunistic and omnivorous like they do in other regions where they consume any type of food available to them. Most cranes concentrated in the northern states of Mexico where food availability is greater than in southern states. Cranes

responded to low availability of corn by moving geographically to a location where corn was available instead of shifting diets.

Sandhill Crane's preference for agricultural grains puts the species in direct conflict with farmers. Sandhill Cranes have become a problem in several regions along their annual cycle due to their crop consumption. This was the first study to investigate the attitudes of rural inhabitants towards Sandhill Cranes at a regional scale in Mexico. My data suggests that farmers in northern Mexico have a good knowledge about the species and cranes are not perceived in a negative way. The majority of local inhabitants that I interviewed did not consider to be affected by the arrival of the cranes. However, with the current population trends and both cranes and farmers having a need for the same resources (i.e., water and crops), conflicts between cranes and humans are expected to rise. In addition, some of the techniques used by farmers to mitigate crop losses can lead to disturbance of Sandhill Cranes and deter them from using already diminishing roosting sites.

Wintering habitat in Mexico may represent a maintenance-type environment that allows cranes to sustain their body weight until they reach the staging grounds. On the one hand, Sandhill Cranes are benefiting from an increase in agricultural practices and are being recorded in new sites but on the other hand, such practices are draining the water resources needed by the cranes. In addition, not only availability but also predictability of both food and water resources in the future will play an important role in the status of the species in the region. Habitat availability has been identified as the most important limiting factor for Sandhill Crane populations. Habitat availability was probably the cause that reduced the historical distribution of the species to winter only in

northern Mexico. Although Sandhill Cranes are extremely adaptable and the Mid-continent Population continues to increase, I predict that in the long term the species will decrease in northern Mexico as habitat conditions degrade due to a combination of water usage and climate change. The Mexican government needs to prioritize wetland conservation as a primary management option to ensure the continuity of the species in the country.

Information gained through this dissertation on the ecology of Sandhill Cranes in Mexico provides valuable insight into how other grassland and waterbirds may respond to increasing threats to their persistence in the region; and therefore, it can serve as a framework for conservation of migratory species in wintering grounds at-large.

# APPENDIX A: MODEL SELECTION OF THE RESULTS OBTAINED FROM LINE TRANSECTS TO ESTIMATE THE DENSITY OF BIRDS OF PREY USING PROGRAM DISTANCE

Table A.1. Model selection for Laguna de San Juan de Ahorcados. Shaded area indicates model selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Uniform | Cosine | 2 | 0.00 | 393.75 | 0.027 | 0.525 | Warning |
| 2 | Negative Exponential | None | 1 | 1.53 | 395.28 | 0.027 | 0.510 | Warning |
| 3 | Hazard-rate | None | 2 | 2.35 | 396.10 | 0.030 | 0.595 | Warning |
| 4 | Half-normal | Cosine | 2 | 4.03 | 397.78 | 0.026 | 0.525 | Warning |

[a]CV = Coefficient of Variation.

Table A.2. Model selection for Laguna de Babícora. Shaded area indicates model/s selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Hazard-rate | Hermite Polynomial | 3 | 0.00 | 589.76 | 0.412 | 0.369 | Warning |
| 2 | Negative Exponential | Simple Polynomial | 3 | 8.25 | 598.00 | 0.361 | 0.272 | Warning |
| 3 | Half-normal | Cosine | 4 | 15.38 | 605.13 | 0.221 | 0.261 | Warning |
| 4 | Uniform | Cosine | 4 | 20.09 | 609.85 | 0.181 | 0.265 | Warning |

[a]CV = Coefficient of Variation.

Table A.3. Model selection for Presa San Carlos de Mapimí. Shaded area indicates model/s selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Negative Exponential | None | 1 | 0.00 | 148.07 | 0.006 | 0.468 | Warning |
| 2 | Uniform | Cosine | 1 | 0.06 | 148.13 | 0.005 | 0.382 | Warning |
| 3 | Hazard-rate | None | 2 | 0.86 | 148.93 | 0.006 | 0.658 | Warning |
| 4 | Half-normal | Cosine | 2 | 0.87 | 148.94 | 0.007 | 0.547 | Warning |

[a]CV = Coefficient of Variation.

Table A.4. Model selection for Laguna de Mexicanos. Shaded area indicates model/s selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Hazard-rate | None | 2 | 0.00 | 2282.57 | 0.091 | 0.102 | Warning |
| 2 | Half-normal | Cosine | 2 | 6.16 | 2288.73 | 0.099 | 0.083 | Warning |
| 3 | Uniform | Cosine | 3 | 12.93 | 2295.51 | 0.086 | 0.088 | Warning |
| 4 | Negative Exponential | None | 1 | 18.45 | 2301.02 | 0.135 | 0.104 | Warning |

[a]CV = Coefficient of Variation.

Table A.5. Model selection for Laguna de Ojo Federico. Shaded area indicates model/s selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Uniform | Cosine | 2 | 0.00 | 204.91 | 0.020 | 0.228 | Warning |
| 2 | Hazard-rate | None | 2 | 0.07 | 204.98 | 0.020 | 0.260 | Warning |
| 3 | Negative Exponential | None | 1 | 0.25 | 205.17 | 0.027 | 0.283 | Warning |
| 4 | Half-normal | None | 1 | 1.02 | 205.94 | 0.018 | 0.136 | Warning |

[a]CV = Coefficient of Variation.

Table A.6. Model selection for Laguna de Santiaguillo. Shaded area indicates model/s selected.

| No. of Models | Standard Models | Adjustments | No. Parameters | ΔAIC | AIC | Density (individuals/ha) | CV[a] | Run Status |
|---|---|---|---|---|---|---|---|---|
| 1 | Negative Exponential | None | 1 | 0.00 | 1129.60 | 0.060 | 0.319 | Warning |
| 2 | Hazard-rate | None | 2 | 2.31 | 1131.92 | 0.042 | 0.322 | Warning |
| 3 | Half-normal | Cosine | 4 | 3.82 | 1133.43 | 0.051 | 0.318 | Warning |
| 4 | Uniform | Cosine | 3 | 8.72 | 1138.32 | 0.036 | 0.308 | Warning |

[a]CV = Coefficient of Variation.

## APPENDIX B: STANDARD CURVES WITH REGRESSION LINES OBTAINED FOR EACH CORTICOSTERONE ELISA KIT

Table B.1. Results obtained for the seven standards of known corticosterone concentration prepared for every corticosterone ELISA kit used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | $\%B/B_0$[c] |
|---|---|---|---|---|---|---|---|
| 1 | $S_0$ | 0 | 1.394 | 1.267 | 1.331 | 1.331 | 100 |
| | $S_1$ | 0.05 | 1.133 | 1.082 | 1.108 | 1.331 | 83 |
| | $S_2$ | 0.1 | 0.996 | 1.071 | 1.034 | 1.331 | 78 |
| | $S_3$ | 0.2 | 0.869 | 0.886 | 0.878 | 1.331 | 66 |
| | $S_4$ | 0.5 | 0.647 | 0.667 | 0.657 | 1.331 | 49 |
| | $S_5$ | 1 | 0.489 | 0.491 | 0.490 | 1.331 | 37 |
| | $S_6$ | 2 | 0.319 | 0.332 | 0.326 | 1.331 | 24 |
| | $S_7$ | 5 | 0.208 | 0.211 | 0.210 | 1.331 | 16 |
| 2 | $S_0$ | 0 | 1.487 | 1.481 | 1.484 | 1.484 | 100 |
| | $S_1$ | 0.05 | 1.340 | 1.316 | 1.328 | 1.484 | 89 |
| | $S_2$ | 0.1 | 1.217 | 1.253 | 1.235 | 1.484 | 83 |
| | $S_3$ | 0.2 | 1.043 | 1.042 | 1.043 | 1.484 | 70 |
| | $S_4$ | 0.5 | 0.788 | 0.745 | 0.767 | 1.484 | 52 |
| | $S_5$ | 1 | 0.585 | 0.564 | 0.575 | 1.484 | 39 |
| | $S_6$ | 2 | 0.405 | 0.395 | 0.400 | 1.484 | 27 |
| | $S_7$ | 5 | 0.241 | 0.253 | 0.247 | 1.484 | 17 |

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 3 | $S_0$ | 0 | 1.631 | 1.855 | 1.743 | 1.743 | 100 |
| | $S_1$ | 0.05 | 1.322 | 1.359 | 1.341 | 1.743 | 77 |
| | $S_2$ | 0.1 | 1.327 | 1.270 | 1.299 | 1.743 | 74 |
| | $S_3$ | 0.2 | 1.117 | 1.098 | 1.108 | 1.743 | 64 |
| | $S_4$ | 0.5 | 0.812 | 0.829 | 0.821 | 1.743 | 47 |
| | $S_5$ | 1 | 0.571 | 0.653 | 0.612 | 1.743 | 35 |
| | $S_6$ | 2 | 0.473 | 0.459 | 0.466 | 1.743 | 27 |
| | $S_7$ | 5 | 0.289 | 0.249 | 0.269 | 1.743 | 15 |
| 4 | $S_0$ | 0 | 1.333 | 1.377 | 1.355 | 1.355 | 100 |
| | $S_1$ | 0.05 | 1.212 | 1.279 | 1.246 | 1.355 | 92 |
| | $S_2$ | 0.1 | 1.098 | 1.171 | 1.135 | 1.355 | 84 |
| | $S_3$ | 0.2 | 0.905 | 0.984 | 0.945 | 1.355 | 70 |
| | $S_4$ | 0.5 | 0.621 | 0.651 | 0.636 | 1.355 | 47 |
| | $S_5$ | 1 | 0.481 | 0.489 | 0.485 | 1.355 | 36 |
| | $S_6$ | 2 | 0.311 | 0.316 | 0.314 | 1.355 | 23 |
| | $S_7$ | 5 | 0.189 | 0.198 | 0.194 | 1.355 | 14 |
| 5 | $S_0$ | 0 | 1.295 | 1.301 | 1.298 | 1.298 | 100 |
| | $S_1$ | 0.05 | 1.215 | 1.127 | 1.171 | 1.298 | 90 |
| | $S_2$ | 0.1 | 1.044 | 0.986 | 1.015 | 1.298 | 78 |
| | $S_3$ | 0.2 | 0.854 | 0.848 | 0.851 | 1.298 | 66 |
| | $S_4$ | 0.5 | 0.562 | 0.618 | 0.590 | 1.298 | 45 |
| | $S_5$ | 1 | 0.425 | 0.444 | 0.435 | 1.298 | 33 |
| | $S_6$ | 2 | 0.336 | 0.328 | 0.332 | 1.298 | 26 |
| | $S_7$ | 5 | 0.202 | 0.196 | 0.199 | 1.298 | 15 |

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 6 | $S_0$ | 0 | 1.299 | 1.348 | 1.324 | 1.324 | 100 |
| | $S_1$ | 0.05 | 1.258 | 1.296 | 1.277 | 1.324 | 96 |
| | $S_2$ | 0.1 | 1.225 | 1.214 | 1.220 | 1.324 | 92 |
| | $S_3$ | 0.2 | 0.894 | 1.045 | 0.970 | 1.324 | 73 |
| | $S_4$ | 0.5 | 0.773 | 0.780 | 0.777 | 1.324 | 59 |
| | $S_5$ | 1 | 0.552 | 0.562 | 0.557 | 1.324 | 42 |
| | $S_6$ | 2 | 0.384 | 0.391 | 0.388 | 1.324 | 29 |
| | $S_7$ | 5 | 0.236 | 0.230 | 0.233 | 1.324 | 18 |
| 7 | $S_0$ | 0 | 1.234 | 1.246 | 1.240 | 1.240 | 100 |
| | $S_1$ | 0.05 | 1.141 | 1.193 | 1.167 | 1.240 | 94 |
| | $S_2$ | 0.1 | 1.214 | 1.267 | 1.241 | 1.240 | 100 |
| | $S_3$ | 0.2 | 1.036 | 1.102 | 1.069 | 1.240 | 86 |
| | $S_4$ | 0.5 | 0.754 | 0.782 | 0.768 | 1.240 | 62 |
| | $S_5$ | 1 | 0.563 | 0.526 | 0.545 | 1.240 | 44 |
| | $S_6$ | 2 | 0.383 | 0.357 | 0.370 | 1.240 | 30 |
| | $S_7$ | 5 | 0.209 | 0.224 | 0.217 | 1.240 | 17 |
| 8 | $S_0$ | 0 | 1.708 | 1.655 | 1.682 | 1.682 | 100 |
| | $S_1$ | 0.05 | 1.520 | 1.480 | 1.500 | 1.682 | 89 |
| | $S_2$ | 0.1 | 1.408 | 1.264 | 1.336 | 1.682 | 79 |
| | $S_3$ | 0.2 | 1.165 | 1.058 | 1.112 | 1.682 | 66 |
| | $S_4$ | 0.5 | 0.830 | 0.775 | 0.803 | 1.682 | 48 |
| | $S_5$ | 1 | 0.604 | 0.536 | 0.570 | 1.682 | 34 |
| | $S_6$ | 2 | 0.400 | 0.363 | 0.382 | 1.682 | 23 |
| | $S_7$ | 5 | 0.237 | 0.225 | 0.231 | 1.682 | 14 |

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 9 | $S_0$ | 0 | 1.613 | 1.513 | 1.563 | 1.563 | 100 |
| | $S_1$ | 0.05 | 1.350 | 1.322 | 1.336 | 1.563 | 85 |
| | $S_2$ | 0.1 | 1.326 | 1.202 | 1.264 | 1.563 | 81 |
| | $S_3$ | 0.2 | 1.119 | 1.086 | 1.103 | 1.563 | 71 |
| | $S_4$ | 0.5 | 0.816 | 0.849 | 0.833 | 1.563 | 53 |
| | $S_5$ | 1 | 0.519 | 0.563 | 0.541 | 1.563 | 35 |
| | $S_6$ | 2 | 0.416 | 0.365 | 0.391 | 1.563 | 25 |
| | $S_7$ | 5 | 0.237 | 0.250 | 0.244 | 1.563 | 16 |
| 10 | $S_0$ | 0 | 1.306 | 1.339 | 1.323 | 1.323 | 100 |
| | $S_1$ | 0.05 | 1.072 | 1.188 | 1.130 | 1.323 | 85 |
| | $S_2$ | 0.1 | 1.087 | 1.122 | 1.105 | 1.323 | 84 |
| | $S_3$ | 0.2 | 0.958 | 0.966 | 0.962 | 1.323 | 73 |
| | $S_4$ | 0.5 | 0.641 | 0.694 | 0.668 | 1.323 | 50 |
| | $S_5$ | 1 | 0.540 | 0.501 | 0.521 | 1.323 | 39 |
| | $S_6$ | 2 | 0.373 | 0.329 | 0.351 | 1.323 | 27 |
| | $S_7$ | 5 | 0.224 | 0.192 | 0.208 | 1.323 | 16 |
| 11 | $S_0$ | 0 | 1.686 | 1.851 | 1.769 | 1.769 | 100 |
| | $S_1$ | 0.05 | 1.643 | 1.747 | 1.695 | 1.769 | 96 |
| | $S_2$ | 0.1 | 1.512 | 1.602 | 1.557 | 1.769 | 88 |
| | $S_3$ | 0.2 | 1.270 | 1.317 | 1.294 | 1.769 | 73 |
| | $S_4$ | 0.5 | 1.008 | 0.996 | 1.002 | 1.769 | 57 |
| | $S_5$ | 1 | 0.766 | 0.739 | 0.753 | 1.769 | 43 |
| | $S_6$ | 2 | 0.546 | 0.509 | 0.528 | 1.769 | 30 |
| | $S_7$ | 5 | 0.360 | 0.295 | 0.328 | 1.769 | 19 |

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 12 | $S_0$ | 0 | 1.837 | 1.769 | 1.803 | 1.803 | 100 |
| | $S_1$ | 0.05 | 1.602 | 1.613 | 1.608 | 1.803 | 89 |
| | $S_2$ | 0.1 | 1.474 | 1.541 | 1.508 | 1.803 | 84 |
| | $S_3$ | 0.2 | 1.311 | 1.303 | 1.307 | 1.803 | 72 |
| | $S_4$ | 0.5 | 1.018 | 1.038 | 1.028 | 1.803 | 57 |
| | $S_5$ | 1 | 0.791 | 0.814 | 0.803 | 1.803 | 45 |
| | $S_6$ | 2 | 0.517 | 0.534 | 0.526 | 1.803 | 29 |
| | $S_7$ | 5 | 0.334 | 0.327 | 0.331 | 1.803 | 18 |
| 13 | $S_0$ | 0 | 1.528 | 1.450 | 1.489 | 1.489 | 100 |
| | $S_1$ | 0.05 | 1.485 | 1.448 | 1.467 | 1.489 | 98 |
| | $S_2$ | 0.1 | 1.394 | 1.194 | 1.294 | 1.489 | 87 |
| | $S_3$ | 0.2 | 1.272 | 1.251 | 1.262 | 1.489 | 85 |
| | $S_4$ | 0.5 | 0.892 | 0.899 | 0.896 | 1.489 | 60 |
| | $S_5$ | 1 | 0.733 | 0.655 | 0.694 | 1.489 | 47 |
| | $S_6$ | 2 | 0.520 | 0.517 | 0.519 | 1.489 | 35 |
| | $S_7$ | 5 | 0.324 | 0.303 | 0.314 | 1.489 | 21 |

[a] OD = Optical Density or Absorbance.

[b] Blank = Average optical density reading of standard with zero concentration of corticosterone.

[c] %B/$B_0$ = Percent of maximal binding or sensitivity. B = Average optical density reading of each standard ($S_1$ = $B_1$ to $S_7$ = $B_7$). $B_0$ = Optical density of blank.

Table B.2. Regression equations calculated for each standard curve obtained for each corticosterone ELISA kit used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in northern Mexico during the winter (October to February) of 2007/08 and 2008/09 (see Fig. B.1 and B.2).

| Kit No. | Regression Equation | $R^2$ | $R^2$ Adjusted |
|---|---|---|---|
| 1 | $\%B/B_0 = 38.5\text{-}36.3 \log ng/g$ | 0.991 | 0.989 |
| 2 | $\%B/B_0 = 41.0\text{-}39.0 \log ng/g$ | 0.992 | 0.990 |
| 3 | $\%B/B_0 = 37.6\text{-}33.2 \log ng/g$ | 0.987 | 0.985 |
| 4 | $\%B/B_0 = 38.5\text{-}41.7 \log ng/g$ | 0.986 | 0.984 |
| 5 | $\%B/B_0 = 37.8\text{-}38.9 \log ng/g$ | 0.987 | 0.984 |
| 6 | $\%B/B_0 = 44.6\text{-}42.3 \log ng/g$ | 0.989 | 0.987 |
| 7 | $\%B/B_0 = 47.3\text{-}44.5 \log ng/g$ | 0.956 | 0.947 |
| 8 | $\%B/B_0 = 37.3\text{-}40.0 \log ng/g$ | 0.991 | 0.989 |
| 9 | $\%B/B_0 = 39.6\text{-}38.4 \log ng/g$ | 0.981 | 0.977 |
| 10 | $\%B/B_0 = 40.8\text{-}38.4 \log ng/g$ | 0.981 | 0.977 |
| 11 | $\%B/B_0 = 44.4\text{-}40.7 \log ng/g$ | 0.995 | 0.994 |
| 12 | $\%B/B_0 = 44.0\text{-}37.6 \log ng/g$ | 0.991 | 0.989 |
| 13 | $\%B/B_0 = 48.6\text{-}40.4 \log ng/g$ | 0.984 | 0.981 |

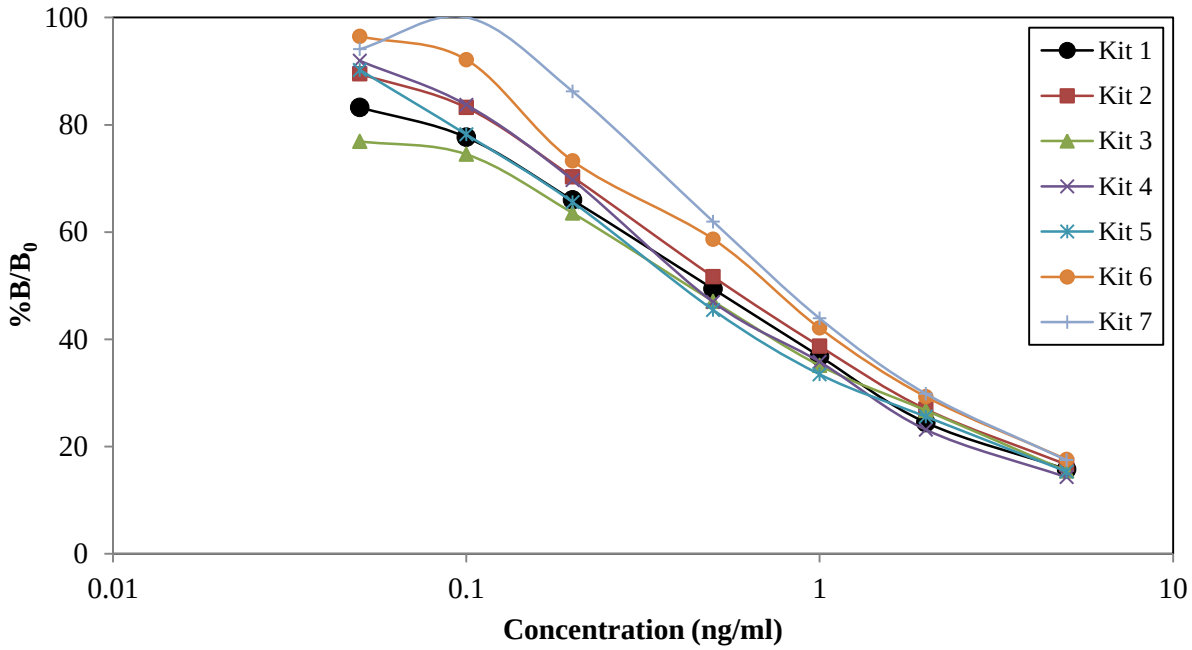


Figure B.1. Standard curves obtained for corticosterone ELISA kits No. 1 to 7 used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

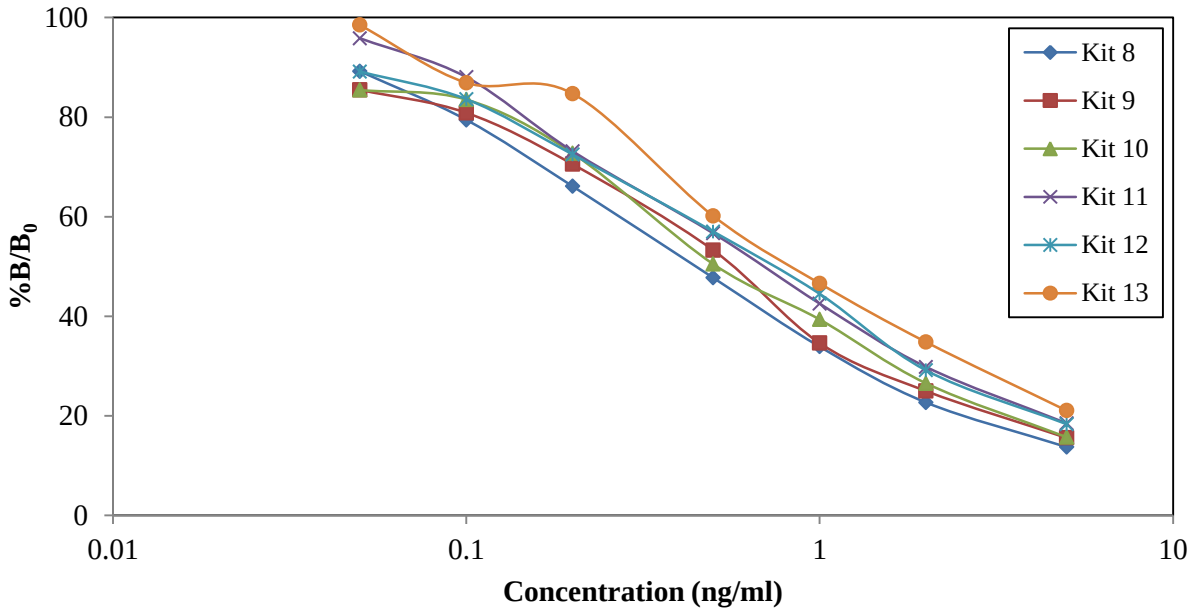


Figure B.2. Standard curves obtained for corticosterone ELISA kits No. 8 to 13 used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in northern Mexico during the winter (October to February) of 2007/08 and 2008/09.

# APPENDIX C: GENERALIZED LINEAR MIXED MODELS NOT SELECTED IN THE ANALYSIS OF FACTORS AFFECTING CORTICOSTERONE LEVELS

Table C.1. List of Generalized Linear Mixed Models (GLMM) with AIC scores above 150. These models were not selected to examine the effects of location to the levels of corticosterone from Sandhill Cranes (*Grus canadensis*) wintering in northern Mexico during 2007 and 2009.

| Model | AIC[a] | ΔAIC[b] | *k*[c] | $w_i$[d] |
|---|---|---|---|---|
| LogCort[e] ~ Month * CityD[f] + (1 \| Wetland) | 152.5 | 123.3 | 10 | < 0.0001 |
| LogCort ~ Month + FoodA[g] + (1 \| Wetland) | 153.6 | 124.4 | 7 | < 0.0001 |
| LogCort ~ Month + BirdsPD[h] + (1 \| Wetland) | 155.7 | 126.5 | 7 | < 0.0001 |
| LogCort ~ Month + (1 \| Wetland) | 155.8 | 126.6 | 6 | < 0.0001 |
| LogCort ~ Month + WetS[i] + (1 \| Wetland) | 155.9 | 126.8 | 7 | < 0.0001 |
| LogCort ~ Month + NCranes[j] + (1 \| Wetland) | 156.6 | 127.4 | 7 | < 0.0001 |
| LogCort ~ Month + CityD + (1 \| Wetland) | 157.3 | 128.1 | 7 | < 0.0001 |
| LogCort ~ Month + WetS + CityD + (1 \| Wetland) | 157.9 | 128.7 | 8 | < 0.0001 |
| LogCort ~ Year * FoodA + (1 \| Wetland) | 174.3 | 145.1 | 6 | < 0.0001 |
| LogCort ~ (Year + WetS + CityD) ^ 2 + (1 \| Wetland) | 176.1 | 146.9 | 9 | < 0.0001 |
| LogCort ~ (Year + WetS + FoodA) ^ 2 + (1 \| Wetland) | 177.7 | 148.5 | 9 | < 0.0001 |
| LogCort ~ Year * WetS + (1 \| Wetland) | 179.1 | 149.9 | 6 | < 0.0001 |
| LogCort ~ Year + FoodA + (1 \| Wetland) | 179.9 | 150.7 | 5 | < 0.0001 |
| LogCort ~ (Year + BirdsPD + CarniD[k]) ^ 2 + (1 \| Wetland) | 181.5 | 152.3 | 9 | < 0.0001 |

| Model | AIC[a] | ΔAIC[b] | $k$[c] | $w_i$[d] |
|---|---|---|---|---|
| LogCort ~ Year + (1 \| Wetland) | 183.7 | 154.6 | 4 | < 0.0001 |
| LogCort ~ Year + WetS + CityD + (1 \| Wetland) | 183.9 | 154.7 | 6 | < 0.0001 |
| LogCort ~ FoodA + (1 \| Wetland) | 228.4 | 199.2 | 4 | < 0.0001 |
| LogCort ~ WetS + (1 \| Wetland) | 230.2 | 201.0 | 4 | < 0.0001 |
| LogCort ~ FoodA + FoodA ^ 2 + (1 \| Wetland) | 230.3 | 201.1 | 5 | < 0.0001 |
| LogCort ~ WetS + WetS ^ 2 + (1 \| Wetland) | 231.4 | 202.2 | 5 | < 0.0001 |
| LogCort ~ BirdsPD + (1 \| Wetland) | 231.4 | 202.3 | 4 | < 0.0001 |
| LogCort ~ FoodA * WetS + (1 \| Wetland) | 232.2 | 203.0 | 6 | < 0.0001 |
| LogCort ~ NCranes + (1 \| Wetland) | 232.5 | 203.3 | 4 | < 0.0001 |
| LogCort ~ CityD + (1 \| Wetland) | 232.6 | 203.4 | 4 | < 0.0001 |
| LogCort ~ CarniD + (1 \| Wetland) | 233.1 | 203.9 | 4 | < 0.0001 |

[a] AIC = Akaike's Information Criterion

[b] ΔAIC = Delta Akaike's Information Criterion

[c] $k$ = Number of parameters

[d] $w_i$ = Weight of the model

[e] LogCort = Log transformed corticosterone concentration (ng/g)

[f] CityD = Distance to city

[g] FoodA = Food abundance

[h] BirdsPD = Density of birds of prey

[i] WetS = Wetland size

[j] NCranes = Number of cranes

[k] CarniD = Density of carnivores

## APPENDIX D: STANDARD CURVES WITH REGRESSION LINES OBTAINED FOR EACH CORTICOSTERONE ELISA KIT

Table D.1. Results obtained for the seven standards of known corticosterone concentration prepared for every corticosterone ELISA kit used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in Nebraska during the spring (March and April) of 2009.

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 14 | $S_0$ | 0 | 1.513 | 1.469 | 1.491 | 1.491 | 100 |
| | $S_1$ | 0.05 | 1.387 | 1.396 | 1.392 | 1.491 | 93 |
| | $S_2$ | 0.1 | 1.220 | 1.365 | 1.293 | 1.491 | 87 |
| | $S_3$ | 0.2 | 1.099 | 1.157 | 1.128 | 1.491 | 76 |
| | $S_4$ | 0.5 | 0.821 | 0.945 | 0.883 | 1.491 | 59 |
| | $S_5$ | 1 | 0.733 | 0.730 | 0.732 | 1.491 | 49 |
| | $S_6$ | 2 | 0.534 | 0.596 | 0.565 | 1.491 | 38 |
| | $S_7$ | 5 | 0.447 | 0.464 | 0.456 | 1.491 | 31 |
| 15 | $S_0$ | 0 | 2.013 | 1.871 | 1.942 | 1.942 | 100 |
| | $S_1$ | 0.05 | 1.772 | 1.904 | 1.838 | 1.942 | 95 |
| | $S_2$ | 0.1 | 1.688 | 1.787 | 1.738 | 1.942 | 89 |
| | $S_3$ | 0.2 | 1.510 | 1.557 | 1.534 | 1.942 | 79 |
| | $S_4$ | 0.5 | 1.222 | 1.216 | 1.219 | 1.942 | 63 |
| | $S_5$ | 1 | 1.015 | 0.944 | 0.980 | 1.942 | 50 |
| | $S_6$ | 2 | 0.750 | 0.709 | 0.730 | 1.942 | 38 |
| | $S_7$ | 5 | 0.561 | 0.543 | 0.552 | 1.942 | 28 |

| Kit No. | Standards | Corticosterone (ng/ml) | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] |
|---|---|---|---|---|---|---|---|
| 16 | $S_0$ | 0 | 2.209 | 1.699 | 1.954 | 1.954 | 100 |
| | $S_1$ | 0.05 | 2.026 | 1.527 | 1.777 | 1.954 | 91 |
| | $S_2$ | 0.1 | 1.856 | 1.574 | 1.715 | 1.954 | 88 |
| | $S_3$ | 0.2 | 1.678 | 1.587 | 1.633 | 1.954 | 84 |
| | $S_4$ | 0.5 | 1.231 | 1.216 | 1.224 | 1.954 | 63 |
| | $S_5$ | 1 | 1.007 | 0.996 | 1.002 | 1.954 | 51 |
| | $S_6$ | 2 | 0.691 | 0.730 | 0.711 | 1.954 | 36 |
| | $S_7$ | 5 | 0.513 | 0.533 | 0.523 | 1.954 | 27 |
| 17 | $S_0$ | 0 | 1.381 | 1.210 | 1.296 | 1.296 | 100 |
| | $S_1$ | 0.05 | 1.133 | 1.119 | 1.126 | 1.296 | 87 |
| | $S_2$ | 0.1 | 1.053 | 0.975 | 1.014 | 1.296 | 78 |
| | $S_3$ | 0.2 | 0.956 | 0.930 | 0.943 | 1.296 | 73 |
| | $S_4$ | 0.5 | 0.728 | 0.686 | 0.707 | 1.296 | 55 |
| | $S_5$ | 1 | 0.703 | 0.662 | 0.683 | 1.296 | 53 |
| | $S_6$ | 2 | 0.543 | 0.503 | 0.523 | 1.296 | 40 |
| | $S_7$ | 5 | 0.462 | 0.412 | 0.437 | 1.296 | 34 |

[a] OD = Optical Density or Absorbance.

[b] Blank = Average optical density reading of standard with zero concentration of corticosterone.

[c] %B/$B_0$ = Percent of maximal binding or sensitivity. B = Average optical density reading of each standard ($S_1$ = $B_1$ to $S_7$ = $B_7$). $B_0$ = Optical density of blank.

Table D.2. Regression equations calculated for each standard curve obtained for each corticosterone ELISA kit used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in Nebraska during the spring (March and April) of 2009 (see Fig. D.1).

| Kit No. | Regression Equation | $R^2$ | $R^2$ Adjusted |
|---|---|---|---|
| 14 | $\%B/B_0 = 50.7\text{-}33.6 \log ng/g$ | 0.991 | 0.989 |
| 15 | $\%B/B_0 = 51.5\text{-}35.6 \log ng/g$ | 0.991 | 0.989 |
| 16 | $\%B/B_0 = 51.1\text{-}35.3 \log ng/g$ | 0.970 | 0.965 |
| 17 | $\%B/B_0 = 50.9\text{-}27.5 \log ng/g$ | 0.983 | 0.979 |

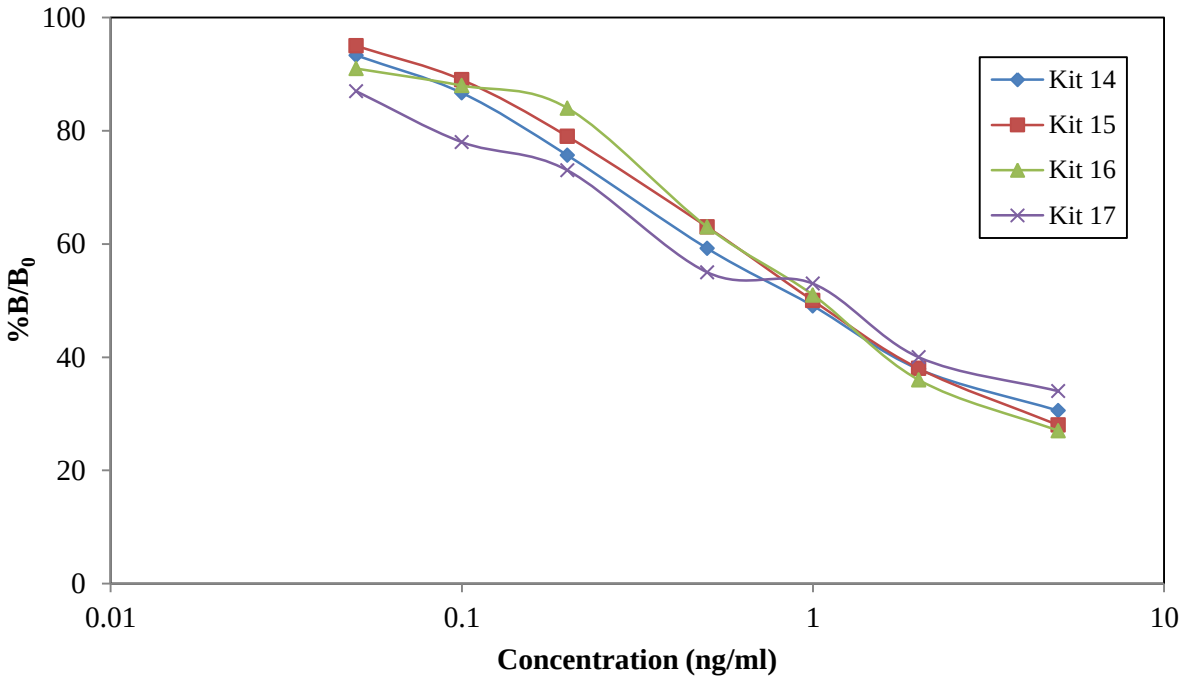


Figure D.1. Standard curves obtained for corticosterone ELISA kits No. 14 to 17 used in the analysis of hormonal extractions attained from Sandhill Cranes (*Grus canadensis*) fecal samples collected in Nebraska during the spring (March and April) of 2009.

## APPENDIX E: CORTICOSTERONE CONCENTRATION CALCULATIONS FOR THE EIA ASSAY

Table E.1. Optical densities obtained from fecal samples from Sandhill Cranes (*Grus canadensis*) collected in Nebraska during the spring (March and April) of 2009 and in Wisconsin during summer and fall (June to November) of 2008. The optical densities were measured using a microplate reader with a 650-nm filter as part of an enzyme immunoassay analysis (EIA) performed to estimate corticosterone concentration. Information regarding the regression equations used in the calculations and obtained for kits 14 to 17 is included in Appendix D.

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-1 | Rowe Sanctuary | 0.657 | 0.628 | 0.643 | 1.491 | 43.092 | 14 | 6.731 | 4.86 | 5.00 | 0.14 | 240.381 |
| IB-2 | Rowe Sanctuary | 1.011 | 1.062 | 1.037 | 1.491 | 69.517 | 14 | 1.102 | 4.84 | 5.07 | 0.23 | 23.950 |
| IB-3 | Rowe Sanctuary | 0.553 | 0.560 | 0.557 | 1.491 | 37.324 | 14 | 10.001 | 4.84 | 5.08 | 0.24 | 208.363 |
| IB-4 | Rowe Sanctuary | 1.224 | 1.100 | 1.162 | 1.296 | 89.695 | 17 | 0.156 | 4.90 | 5.23 | 0.33 | 2.364 |
| IB-5 | Rowe Sanctuary | 1.077 | 1.167 | 1.122 | 1.491 | 75.252 | 14 | 0.743 | 4.68 | 4.93 | 0.25 | 14.862 |
| IB-6 | Rowe Sanctuary | 1.217 | 1.324 | 1.271 | 1.491 | 85.211 | 14 | 0.376 | 4.69 | 5.05 | 0.36 | 5.221 |
| IB-7 | Rowe Sanctuary | 0.882 | 0.903 | 0.893 | 1.491 | 59.859 | 14 | 2.133 | 4.80 | 4.95 | 0.15 | 71.111 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-8 | Corn Field | 0.847 | 0.912 | 0.880 | 1.491 | 58.987 | 14 | 2.265 | 4.73 | 4.87 | 0.14 | 80.891 |
| IB-9 | Trust S. Blind | 0.749 | 0.731 | 0.740 | 1.491 | 49.631 | 14 | 4.306 | 4.70 | 4.97 | 0.27 | 79.739 |
| IB-10 | Rowe Sanctuary | 1.035 | 1.119 | 1.077 | 1.491 | 72.233 | 14 | 0.914 | 4.76 | 5.04 | 0.28 | 16.326 |
| IB-11 | Trust S. Blind | 1.249 | 1.244 | 1.247 | 1.491 | 83.602 | 14 | 0.420 | 4.84 | 5.11 | 0.27 | 7.774 |
| IB-12 | Corn Field | 0.802 | 0.867 | 0.835 | 1.491 | 55.969 | 14 | 2.787 | 4.74 | 4.90 | 0.16 | 87.079 |
| IB-13 | Trust S. Blind | 1.166 | 1.231 | 1.199 | 1.491 | 80.382 | 14 | 0.524 | 4.70 | 4.97 | 0.27 | 9.698 |
| IB-14 | Trust S. Blind | 1.069 | 1.080 | 1.075 | 1.491 | 72.066 | 14 | 0.925 | 4.77 | 5.00 | 0.23 | 20.105 |
| IB-15 | Trust S. Blind | 1.021 | 1.075 | 1.048 | 1.491 | 70.288 | 14 | 1.045 | 4.76 | 4.96 | 0.20 | 26.122 |
| IB-16 | Trust S. Blind | 0.707 | 0.734 | 0.721 | 1.491 | 48.323 | 14 | 4.710 | 4.76 | 4.98 | 0.22 | 107.055 |
| IB-17 | Trust S. Blind | 1.237 | 1.184 | 1.211 | 1.491 | 81.187 | 14 | 0.496 | 4.77 | 5.02 | 0.25 | 9.910 |
| IB-18 | Trust S. Blind | 1.141 | 1.146 | 1.144 | 1.491 | 76.693 | 14 | 0.673 | 4.82 | 5.11 | 0.29 | 11.605 |
| IB-19 | Rowe Sanctuary | 1.060 | 1.049 | 1.055 | 1.491 | 70.724 | 14 | 1.014 | 4.77 | 5.08 | 0.31 | 16.355 |
| IB-20 | Trust S. Blind | 0.821 | 0.757 | 0.789 | 1.491 | 52.918 | 14 | 3.436 | 4.77 | 4.98 | 0.21 | 81.810 |
| IB-21 | Trust S. Blind | 1.276 | 1.344 | 1.310 | 1.942 | 67.456 | 15 | 1.426 | 4.83 | 5.03 | 0.20 | 35.645 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-22 | Trust S. Blind | 1.228 | 1.265 | 1.247 | 1.942 | 64.186 | 15 | 1.762 | 4.85 | 5.13 | 0.28 | 31.468 |
| IB-23 | Trust S. Blind | 1.777 | 1.863 | 1.820 | 1.942 | 93.718 | 15 | 0.261 | 4.77 | 5.11 | 0.34 | 3.833 |
| IB-24 | Trust S. Blind | 1.258 | 1.350 | 1.304 | 1.942 | 67.147 | 15 | 1.452 | 4.75 | 5.11 | 0.36 | 20.171 |
| IB-25 | Rowe Sanctuary | 1.132 | 1.151 | 1.142 | 1.942 | 58.780 | 15 | 2.501 | 4.92 | 5.10 | 0.18 | 69.463 |
| IB-26 | Mormon Island | 1.198 | 1.163 | 1.181 | 1.942 | 60.788 | 15 | 2.193 | 4.75 | 4.93 | 0.18 | 60.920 |
| IB-27 | Mormon Island | 1.028 | 1.022 | 1.025 | 1.942 | 52.781 | 15 | 3.682 | 4.86 | 5.06 | 0.20 | 92.045 |
| IB-28 | Mormon Island | 1.152 | 1.103 | 1.128 | 1.942 | 58.059 | 15 | 2.619 | 4.69 | 4.90 | 0.21 | 62.347 |
| IB-29 | Mormon Island | 1.122 | 1.146 | 1.134 | 1.942 | 58.393 | 15 | 2.559 | 4.78 | 5.00 | 0.22 | 58.157 |
| IB-30 | Mormon Island | 1.217 | 1.228 | 1.223 | 1.942 | 62.951 | 15 | 1.906 | 4.76 | 4.93 | 0.17 | 56.051 |
| IB-31 | Mormon Island | 1.084 | 1.125 | 1.105 | 1.942 | 56.874 | 15 | 2.825 | 4.92 | 5.10 | 0.18 | 78.480 |
| IB-32 | Mormon Island | 1.065 | 1.111 | 1.088 | 1.942 | 56.025 | 15 | 2.986 | 5.11 | 5.34 | 0.23 | 64.909 |
| IB-33 | Mormon Island | 1.177 | 1.265 | 1.221 | 1.942 | 62.873 | 15 | 1.919 | 4.82 | 5.07 | 0.25 | 38.378 |
| IB-34 | Mormon Island | 1.264 | 1.325 | 1.295 | 1.942 | 66.658 | 15 | 1.500 | 4.80 | 4.98 | 0.18 | 41.663 |
| IB-35 | Mormon Island | 1.060 | 1.058 | 1.059 | 1.942 | 54.531 | 15 | 3.289 | 4.75 | 4.92 | 0.17 | 96.734 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-36 | Mormon Island | 1.193 | 1.261 | 1.227 | 1.942 | 63.182 | 15 | 1.880 | 4.77 | 4.93 | 0.16 | 58.736 |
| IB-37 | Mormon Island | 1.038 | 0.972 | 1.005 | 1.942 | 51.751 | 15 | 3.936 | 4.85 | 5.03 | 0.18 | 109.334 |
| IB-38 | Mormon Island | 1.126 | 1.077 | 1.102 | 1.942 | 56.720 | 15 | 2.851 | 5.11 | 5.38 | 0.27 | 52.804 |
| IB-39 | Mormon Island | 0.808 | 0.813 | 0.811 | 1.942 | 41.735 | 15 | 7.517 | 4.78 | 5.02 | 0.24 | 156.610 |
| IB-40 | Mormon Island | 1.193 | 1.079 | 1.136 | 1.942 | 58.496 | 15 | 2.541 | 4.74 | 4.91 | 0.17 | 74.745 |
| IB-41 | Mormon Island | 1.165 | 1.146 | 1.156 | 1.954 | 59.135 | 16 | 2.366 | 4.77 | 4.90 | 0.13 | 91.009 |
| IB-42 | Mormon Island | 1.356 | 1.342 | 1.349 | 1.954 | 69.038 | 16 | 1.242 | 4.85 | 5.00 | 0.15 | 41.395 |
| IB-43 | Mormon Island | 1.324 | 1.288 | 1.306 | 1.954 | 66.837 | 16 | 1.432 | 4.74 | 4.93 | 0.19 | 37.695 |
| IB-44 | Mormon Island | 0.840 | 0.804 | 0.822 | 1.954 | 42.068 | 16 | 7.212 | 4.76 | 5.03 | 0.27 | 133.557 |
| IB-45 | Mormon Island | 1.256 | 1.274 | 1.265 | 1.954 | 64.739 | 16 | 1.645 | 4.77 | 4.95 | 0.18 | 45.683 |
| IB-46 | Mormon Island | 1.086 | 1.123 | 1.105 | 1.954 | 56.525 | 16 | 2.806 | 4.78 | 5.02 | 0.24 | 58.455 |
| IB-47 | Mormon Island | 1.279 | 1.242 | 1.261 | 1.954 | 64.509 | 16 | 1.667 | 4.71 | 4.91 | 0.20 | 41.687 |
| IB-48 | Mormon Island | 1.152 | 1.126 | 1.139 | 1.954 | 58.291 | 16 | 2.501 | 4.77 | 4.94 | 0.17 | 73.549 |
| IB-49 | Mormon Island | 1.243 | 1.230 | 1.237 | 1.954 | 63.280 | 16 | 1.807 | 4.79 | 5.01 | 0.22 | 41.078 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-50 | Mormon Island | 0.897 | 0.902 | 0.900 | 1.296 | 69.433 | 17 | 0.848 | 4.74 | 4.80 | 0.06 | NA |
| IB-51 | Mormon Island | 1.413 | 1.402 | 1.408 | 1.954 | 72.032 | 16 | 1.021 | 4.71 | 4.93 | 0.22 | 23.206 |
| IB-52 | Mormon Island | 1.405 | 1.312 | 1.359 | 1.954 | 69.524 | 16 | 1.202 | 4.69 | 4.91 | 0.22 | 27.328 |
| IB-53 | Mormon Island | 1.112 | 1.159 | 1.136 | 1.954 | 58.112 | 16 | 2.530 | 4.76 | 4.97 | 0.21 | 60.230 |
| IB-54 | Mormon Island | 1.080 | 1.021 | 1.051 | 1.954 | 53.762 | 16 | 3.366 | 4.75 | 5.00 | 0.25 | 67.312 |
| IB-55 | Mormon Island | 1.055 | 1.027 | 1.041 | 1.954 | 53.275 | 16 | 3.468 | 4.91 | 5.10 | 0.19 | 91.259 |
| IB-56 | Mormon Island | 1.479 | 1.519 | 1.499 | 1.954 | 76.714 | 16 | 0.752 | 4.79 | 4.96 | 0.17 | 22.109 |
| IB-57 | Mormon Island | 0.942 | 0.961 | 0.952 | 1.954 | 48.695 | 16 | 4.678 | 4.90 | 5.11 | 0.21 | 111.381 |
| IB-58 | Mormon Island | 1.027 | 1.038 | 1.033 | 1.954 | 52.840 | 16 | 3.573 | 4.71 | 4.95 | 0.24 | 74.442 |
| IB-59 | Mormon Island | 1.090 | 1.074 | 1.082 | 1.954 | 55.374 | 16 | 3.027 | 4.83 | 5.05 | 0.22 | 68.803 |
| IB-60 | Mormon Island | 1.228 | 1.139 | 1.184 | 1.954 | 60.568 | 16 | 2.158 | 4.80 | 5.00 | 0.20 | 53.951 |
| IB-61 | Mormon Island | 1.249 | 1.174 | 1.212 | 1.954 | 62.001 | 16 | 1.964 | 4.70 | 4.88 | 0.18 | 54.546 |
| IB-62 | Trust Loafing Area | 1.007 | 0.970 | 0.989 | 1.954 | 50.589 | 16 | 4.131 | 4.71 | 4.94 | 0.23 | 89.805 |
| IB-63 | Trust Loafing Area | 1.220 | 1.187 | 1.204 | 1.954 | 61.592 | 16 | 2.019 | 4.76 | 4.90 | 0.14 | 72.094 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-64 | Trust Loafing Area | 1.282 | 1.223 | 1.253 | 1.296 | 96.681 | 17 | 1.082 | 4.70 | 4.80 | 0.10 | 54.075 |
| IB-65 | Trust Loafing Area | 1.006 | 0.962 | 0.984 | 1.954 | 50.358 | 16 | 4.198 | 4.70 | 4.94 | 0.24 | 87.462 |
| IB-66 | Trust Loafing Area | 1.500 | 1.395 | 1.448 | 1.954 | 74.079 | 16 | 0.893 | 4.82 | 5.00 | 0.18 | 24.818 |
| IB-67 | Trust Loafing Area | 1.258 | 1.196 | 1.227 | 1.954 | 62.794 | 16 | 1.867 | 5.12 | 5.32 | 0.20 | 46.666 |
| IB-68 | Trust Loafing Area | 1.266 | 1.198 | 1.232 | 1.954 | 63.050 | 16 | 1.833 | 4.69 | 4.91 | 0.22 | 41.649 |
| IB-69 | Trust Loafing Area | 0.809 | 0.785 | 0.797 | 1.954 | 40.788 | 16 | 7.835 | 4.73 | 4.95 | 0.22 | 178.076 |
| IB-70 | Trust Loafing Area | 1.123 | 1.065 | 1.094 | 1.954 | 55.988 | 16 | 2.911 | 4.74 | 4.95 | 0.21 | 69.312 |
| IB-71 | Trust Loafing Area | 0.474 | 0.484 | 0.479 | 1.296 | 36.974 | 17 | 12.825 | 4.73 | 4.94 | 0.21 | 305.359 |
| IB-72 | Trust Loafing Area | 0.886 | 0.915 | 0.901 | 1.296 | 69.510 | 17 | 0.842 | 4.75 | 4.95 | 0.20 | 21.038 |
| IB-73 | Trust Loafing Area | 0.800 | 0.742 | 0.771 | 1.296 | 59.514 | 17 | 1.946 | 4.69 | 4.87 | 0.18 | 54.046 |
| IB-74 | Trust Loafing Area | 0.838 | 0.844 | 0.841 | 1.296 | 64.917 | 17 | 1.236 | 4.73 | 4.94 | 0.21 | 29.431 |
| IB-75 | Trust Loafing Area | 0.903 | 0.877 | 0.890 | 1.296 | 68.699 | 17 | 0.902 | 4.73 | 5.00 | 0.27 | 16.698 |
| IB-76 | Trust Loafing Area | 0.907 | 0.892 | 0.900 | 1.296 | 69.433 | 17 | 0.847 | 4.74 | 4.90 | 0.16 | 26.480 |
| IB-77 | Trust Loafing Area | 0.907 | 0.971 | 0.939 | 1.296 | 72.482 | 17 | 0.656 | 4.70 | 4.87 | 0.17 | 19.301 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-78 | Trust Loafing Area | 0.756 | 0.821 | 0.789 | 1.296 | 60.865 | 17 | 1.738 | 4.76 | 4.93 | 0.17 | 51.119 |
| IB-79 | Trust Loafing Area | 0.718 | 0.723 | 0.721 | 1.296 | 55.616 | 17 | 2.698 | 4.70 | 4.92 | 0.22 | 61.321 |
| IB-80 | Trust Loafing Area | 1.012 | 1.052 | 1.032 | 1.296 | 79.660 | 17 | 0.360 | 4.80 | 4.97 | 0.17 | 10.582 |
| IB-81 | Trust Loafing Area | 0.864 | 0.886 | 0.875 | 1.296 | 67.541 | 17 | 0.993 | 4.73 | 4.94 | 0.21 | 23.649 |
| IB-82 | Corn Field | 0.702 | 0.782 | 0.742 | 1.296 | 57.275 | 17 | 2.345 | 4.74 | 4.99 | 0.25 | 46.891 |
| IB-83 | Corn Field | 0.858 | 0.911 | 0.885 | 1.296 | 68.275 | 17 | 0.933 | 4.70 | 4.90 | 0.20 | 23.335 |
| IB-84 | Corn Field | 0.834 | 0.849 | 0.842 | 1.296 | 64.956 | 17 | 1.233 | 5.11 | 5.30 | 0.19 | 32.455 |
| IB-85 | Corn Field | 0.849 | 0.867 | 0.858 | 1.296 | 66.229 | 17 | 1.109 | 4.81 | 5.80 | 0.99 | 5.603 |
| IB-86 | Corn Field | 0.767 | 0.786 | 0.777 | 1.296 | 59.938 | 17 | 1.875 | 4.72 | 4.95 | 0.23 | 40.766 |
| IB-87 | Corn Field | 0.891 | 0.812 | 0.852 | 1.296 | 65.728 | 17 | 1.156 | 4.76 | 4.95 | 0.19 | 30.428 |
| IB-88 | Corn Field | 1.040 | 1.000 | 1.020 | 1.296 | 78.734 | 17 | 0.389 | 4.91 | 5.18 | 0.27 | 7.205 |
| IB-89 | Corn Field | 0.710 | 0.656 | 0.683 | 1.296 | 52.721 | 17 | 3.436 | 4.77 | 4.95 | 0.18 | 95.446 |
| IB-90 | Corn Field | 1.044 | 0.976 | 1.010 | 1.296 | 77.962 | 17 | 0.415 | 4.76 | 5.00 | 0.24 | 8.646 |
| IB-91 | Corn Field | 1.063 | 1.006 | 1.035 | 1.296 | 79.853 | 17 | 0.354 | 4.74 | 4.90 | 0.16 | 11.064 |
| IB-92 | Corn Field | 1.000 | 0.928 | 0.964 | 1.296 | 74.411 | 17 | 0.559 | 4.76 | 5.08 | 0.32 | 8.727 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-93 | Corn Field | 0.931 | 0.870 | 0.901 | 1.296 | 69.510 | 17 | 0.842 | 4.75 | 5.02 | 0.27 | 15.584 |
| IB-94 | Corn Field | 1.121 | 1.088 | 1.105 | 1.296 | 85.257 | 17 | 0.225 | 4.72 | 4.90 | 0.18 | 6.262 |
| IB-95 | Corn Field | 0.818 | 0.830 | 0.824 | 1.296 | 63.605 | 17 | 1.381 | 4.85 | 5.06 | 0.21 | 32.870 |
| IB-96 | Corn Field | 1.058 | 1.037 | 1.048 | 1.296 | 80.857 | 17 | 0.326 | 4.83 | 4.98 | 0.15 | 10.863 |
| IB-97 | Corn Field | 1.042 | 1.063 | 1.053 | 1.296 | 81.243 | 17 | 0.316 | 4.66 | 4.87 | 0.21 | 7.513 |
| IB-98 | Corn Field | 1.062 | 1.013 | 1.038 | 1.296 | 80.085 | 17 | 0.348 | 4.69 | 4.90 | 0.21 | 8.276 |
| IB-99 | Corn Field | 0.896 | 0.896 | 0.896 | 1.296 | 69.162 | 17 | 0.867 | 4.79 | 5.02 | 0.23 | 18.850 |
| IB-100 | Corn Field | 0.975 | 0.928 | 0.952 | 1.296 | 73.447 | 17 | 0.605 | 4.73 | 4.95 | 0.22 | 13.760 |
| IB-301 | Rowe Sanctuary | 0.711 | 0.701 | 0.706 | 1.491 | 47.351 | 14 | 5.036 | 4.77 | 4.90 | 0.13 | 96.841 |
| IB-302 | Rowe Sanctuary | 0.991 | 1.000 | 0.996 | 1.491 | 66.767 | 14 | 1.331 | 4.73 | 5.00 | 0.27 | 12.321 |
| IB-303 | Rowe Sanctuary | 0.675 | 0.626 | 0.651 | 1.491 | 43.628 | 14 | 6.487 | 4.69 | 4.89 | 0.20 | 81.091 |
| IB-304 | Rowe Sanctuary | 1.114 | 1.103 | 1.109 | 1.491 | 74.346 | 14 | 0.791 | 4.70 | 5.08 | 0.38 | 5.203 |
| IB-305 | Rowe Sanctuary | 1.179 | 1.118 | 1.149 | 1.491 | 77.029 | 14 | 0.658 | 4.84 | 5.08 | 0.24 | 6.852 |
| IB-306 | Rowe Sanctuary | 1.354 | 1.305 | 1.330 | 1.491 | 89.168 | 14 | 0.286 | 4.78 | 5.10 | 0.32 | 2.238 |
| IB-307 | Rowe Sanctuary | 0.882 | 0.855 | 0.869 | 1.491 | 58.249 | 14 | 2.383 | 4.80 | 4.94 | 0.14 | 42.547 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-308 | Corn Field | 0.937 | 0.833 | 0.885 | 1.491 | 59.356 | 14 | 2.208 | 4.79 | 4.98 | 0.19 | 29.057 |
| IB-309 | Trust S. Blind | 0.741 | 0.722 | 0.732 | 1.491 | 49.061 | 14 | 4.478 | 4.76 | 5.01 | 0.25 | 44.778 |
| IB-310 | Rowe Sanctuary | 1.234 | 1.189 | 1.212 | 1.491 | 81.254 | 14 | 0.493 | 4.70 | 5.01 | 0.31 | 3.978 |
| IB-311 | Trust S. Blind | 1.291 | 1.262 | 1.277 | 1.491 | 85.614 | 14 | 0.366 | 4.75 | 5.04 | 0.29 | 3.152 |
| IB-312 | Corn Field | 0.770 | 0.795 | 0.783 | 1.296 | 60.401 | 17 | 1.803 | 4.80 | 4.94 | 0.14 | 64.403 |
| IB-313 | Trust S. Blind | 1.147 | 1.093 | 1.120 | 1.491 | 75.117 | 14 | 0.750 | 4.77 | 5.08 | 0.31 | 6.048 |
| IB-314 | Trust S. Blind | 1.062 | 0.994 | 1.028 | 1.491 | 68.947 | 14 | 1.146 | 4.74 | 4.95 | 0.21 | 13.639 |
| IB-315 | Trust S. Blind | 0.977 | 0.997 | 0.987 | 1.491 | 66.197 | 14 | 1.384 | 4.77 | 5.01 | 0.24 | 14.414 |
| IB-316 | Trust S. Blind | 0.799 | 0.743 | 0.771 | 1.491 | 51.710 | 14 | 3.733 | 4.77 | 5.01 | 0.24 | 38.885 |
| IB-317 | Trust S. Blind | 1.185 | 1.132 | 1.159 | 1.491 | 77.700 | 14 | 0.628 | 4.84 | 5.06 | 0.22 | 7.138 |
| IB-318 | Trust S. Blind | 1.034 | 0.971 | 1.003 | 1.491 | 67.237 | 14 | 1.288 | 4.70 | 4.94 | 0.24 | 13.421 |
| IB-319 | Rowe Sanctuary | 0.969 | 1.003 | 0.986 | 1.491 | 66.130 | 14 | 1.390 | 4.79 | 5.13 | 0.34 | 10.222 |
| IB-320 | Trust S. Blind | 0.970 | 0.949 | 0.960 | 1.491 | 64.353 | 14 | 1.571 | 4.73 | 5.02 | 0.29 | 13.539 |
| IB-321 | Trust S. Blind | 1.338 | 1.268 | 1.303 | 1.942 | 67.096 | 15 | 1.459 | 4.91 | 5.17 | 0.26 | 14.029 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-322 | Trust S. Blind | 1.225 | 1.192 | 1.209 | 1.942 | 62.230 | 15 | 2.000 | 4.70 | 4.99 | 0.29 | 17.242 |
| IB-323 | Trust S. Blind | 1.776 | 1.587 | 1.682 | 1.942 | 86.586 | 15 | 0.413 | 5.11 | 5.50 | 0.39 | 2.648 |
| IB-324 | Trust S. Blind | 1.181 | 1.161 | 1.171 | 1.942 | 60.299 | 15 | 2.265 | 4.70 | 5.05 | 0.35 | 16.178 |
| IB-325 | Rowe Sanctuary | 0.980 | 0.983 | 0.982 | 1.296 | 75.762 | 17 | 0.499 | 4.73 | 4.80 | 0.07 | 35.640 |
| IB-326 | Mormon Island | 1.070 | 1.108 | 1.089 | 1.942 | 56.076 | 15 | 2.972 | 4.71 | 4.87 | 0.16 | 46.439 |
| IB-327 | Mormon Island | 0.972 | 1.008 | 0.990 | 1.942 | 50.978 | 15 | 4.141 | 4.77 | 4.97 | 0.20 | 51.757 |
| IB-328 | Mormon Island | 0.969 | 0.952 | 0.961 | 1.942 | 49.459 | 15 | 4.561 | 4.81 | 5.01 | 0.20 | 57.012 |
| IB-329 | Mormon Island | 1.037 | 1.066 | 1.052 | 1.942 | 54.145 | 15 | 3.373 | 4.69 | 4.86 | 0.17 | 49.608 |
| IB-330 | Mormon Island | 1.153 | 1.178 | 1.166 | 1.942 | 60.015 | 15 | 2.307 | 4.76 | 4.87 | 0.11 | 52.434 |
| IB-331 | Mormon Island | 0.918 | 0.815 | 0.867 | 1.296 | 66.885 | 17 | 1.050 | 4.75 | 4.90 | 0.15 | 34.989 |
| IB-332 | Mormon Island | 1.062 | 1.100 | 1.081 | 1.942 | 55.664 | 15 | 3.055 | 5.12 | 5.26 | 0.14 | 54.560 |
| IB-333 | Mormon Island | 1.039 | 1.036 | 1.038 | 1.942 | 53.424 | 15 | 3.532 | 4.84 | 5.09 | 0.25 | 35.323 |
| IB-334 | Mormon Island | 0.945 | 0.995 | 0.970 | 1.942 | 49.949 | 15 | 4.426 | 4.76 | 4.95 | 0.19 | 58.243 |
| IB-335 | Mormon Island | 0.863 | 0.972 | 0.918 | 1.942 | 47.245 | 15 | 5.273 | 4.83 | 5.01 | 0.18 | 73.237 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-336 | Mormon Island | 0.993 | 1.122 | 1.058 | 1.942 | 54.454 | 15 | 3.304 | 4.76 | 4.92 | 0.16 | 51.628 |
| IB-337 | Mormon Island | 0.950 | 1.092 | 1.021 | 1.942 | 52.575 | 15 | 3.733 | 5.11 | 5.25 | 0.14 | 66.661 |
| IB-338 | Mormon Island | 1.125 | 1.196 | 1.161 | 1.942 | 59.758 | 15 | 2.345 | 4.84 | 5.08 | 0.24 | 24.423 |
| IB-339 | Mormon Island | 0.853 | 0.852 | 0.853 | 1.942 | 43.898 | 15 | 6.547 | 4.70 | 4.87 | 0.17 | 96.284 |
| IB-340 | Mormon Island | 1.029 | 1.079 | 1.054 | 1.942 | 54.274 | 15 | 3.342 | 4.69 | 4.87 | 0.18 | 46.422 |
| IB-341 | Mormon Island | 1.045 | 1.017 | 1.031 | 1.954 | 52.764 | 16 | 3.590 | 4.78 | 4.95 | 0.17 | 52.790 |
| IB-342 | Mormon Island | 0.900 | 0.864 | 0.882 | 1.296 | 68.082 | 17 | 0.949 | 4.72 | 4.83 | 0.11 | 43.116 |
| IB-343 | Mormon Island | 1.253 | 1.204 | 1.229 | 1.954 | 62.871 | 16 | 1.858 | 4.76 | 4.93 | 0.17 | 27.325 |
| IB-344 | Mormon Island | 0.690 | 0.576 | 0.633 | 1.296 | 48.861 | 17 | 4.743 | 4.81 | 5.00 | 0.19 | 124.818 |
| IB-345 | Mormon Island | 1.463 | 1.390 | 1.427 | 1.954 | 73.004 | 16 | 0.957 | 5.10 | 5.19 | 0.09 | 26.592 |
| IB-346 | Mormon Island | 1.117 | 1.074 | 1.096 | 1.954 | 56.064 | 16 | 2.891 | 4.81 | 5.05 | 0.24 | 30.115 |
| IB-347 | Mormon Island | 1.098 | 1.090 | 1.094 | 1.954 | 55.988 | 16 | 2.911 | 4.73 | 4.92 | 0.19 | 38.304 |
| IB-348 | Mormon Island | 0.958 | 0.972 | 0.965 | 1.954 | 49.386 | 16 | 4.478 | 4.76 | 4.94 | 0.18 | 62.191 |
| IB-349 | Mormon Island | 1.171 | 1.142 | 1.157 | 1.954 | 59.186 | 16 | 2.361 | 4.71 | 4.82 | 0.11 | 53.655 |

| Sample ID | Location | OD[a] Reading 1 | OD Reading 2 | Mean OD Reading | Blank[b] OD Reading | %B/$B_0$[c] | Kit No. | Cort. (ng/ml) | Empty Vial Weight (g) | Dry Vial Weight (g) | Dry Feces Weight (g) | Cort. (ng/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IB-350 | Mormon Island | 1.097 | 1.084 | 1.091 | 1.954 | 55.809 | 16 | 2.945 | 4.69 | 4.92 | 0.23 | 32.009 |
| 17258 | Wisconsin | 0.473 | 0.473 | 0.473 | 1.682 | 28.130 | 8 | 6.777 | 4.73 | 5.36 | 0.63 | 53.789 |
| 17267 | Wisconsin | 0.659 | 0.664 | 0.662 | 1.682 | 39.340 | 8 | 3.557 | 4.90 | 5.95 | 1.05 | 16.937 |
| 17268 | Wisconsin | 0.852 | 0.814 | 0.833 | 1.682 | 49.539 | 8 | 1.977 | 4.78 | 5.78 | 1.00 | 9.886 |
| 17322 | Wisconsin | 0.501 | 0.494 | 0.498 | 1.682 | 29.587 | 8 | 6.238 | 4.84 | 5.45 | 0.61 | 51.133 |
| 17326 | Wisconsin | 0.515 | 0.501 | 0.508 | 1.682 | 30.211 | 8 | 6.013 | 4.86 | 5.13 | 0.27 | 111.344 |
| 17328 | Wisconsin | 0.469 | 0.458 | 0.464 | 1.682 | 27.565 | 8 | 6.999 | 4.77 | 5.21 | 0.44 | 79.539 |
| 17331 | Wisconsin | 0.399 | 0.380 | 0.390 | 1.682 | 23.164 | 8 | 9.017 | 4.83 | 5.20 | 0.37 | 121.851 |
| 17333 | Wisconsin | 0.403 | 0.391 | 0.397 | 1.682 | 23.610 | 8 | 8.791 | 4.69 | 5.05 | 0.36 | 122.103 |
| 17334 | Wisconsin | 0.363 | 0.335 | 0.349 | 1.682 | 20.755 | 8 | 10.377 | 4.87 | 5.18 | 0.31 | 167.366 |
| 17338 | Wisconsin | 0.231 | 0.207 | 0.219 | 1.682 | 13.024 | 8 | 16.183 | 4.76 | 4.97 | 0.21 | 385.310 |

[a] OD = Optical Density or Absorbance.

[b] Blank = Average optical density reading of standard with zero concentration of corticosterone (Appendix B and D).

[c] %B/$B_0$ = Percent of maximal binding or sensitivity where B = Sample average optical density reading and $B_0$ = Average optical density of blank.

# APPENDIX F: SPECIES OF WILD PLANTS FOUND IN FECAL SAMPLES OF SANDHILL CRANES

Figure F.1. Nut-grass (*Cyperus rotundus*). Photo by Heike Vibrans. Public image from <http://www.conabio.gob.mx/invasoras> and used for educational purposes.


Figure F.2. Feather fingergrass (*Chloris virgata*). Photo by University of Arizona. Public image from <http://www.itis.gov> and used for educational purposes.

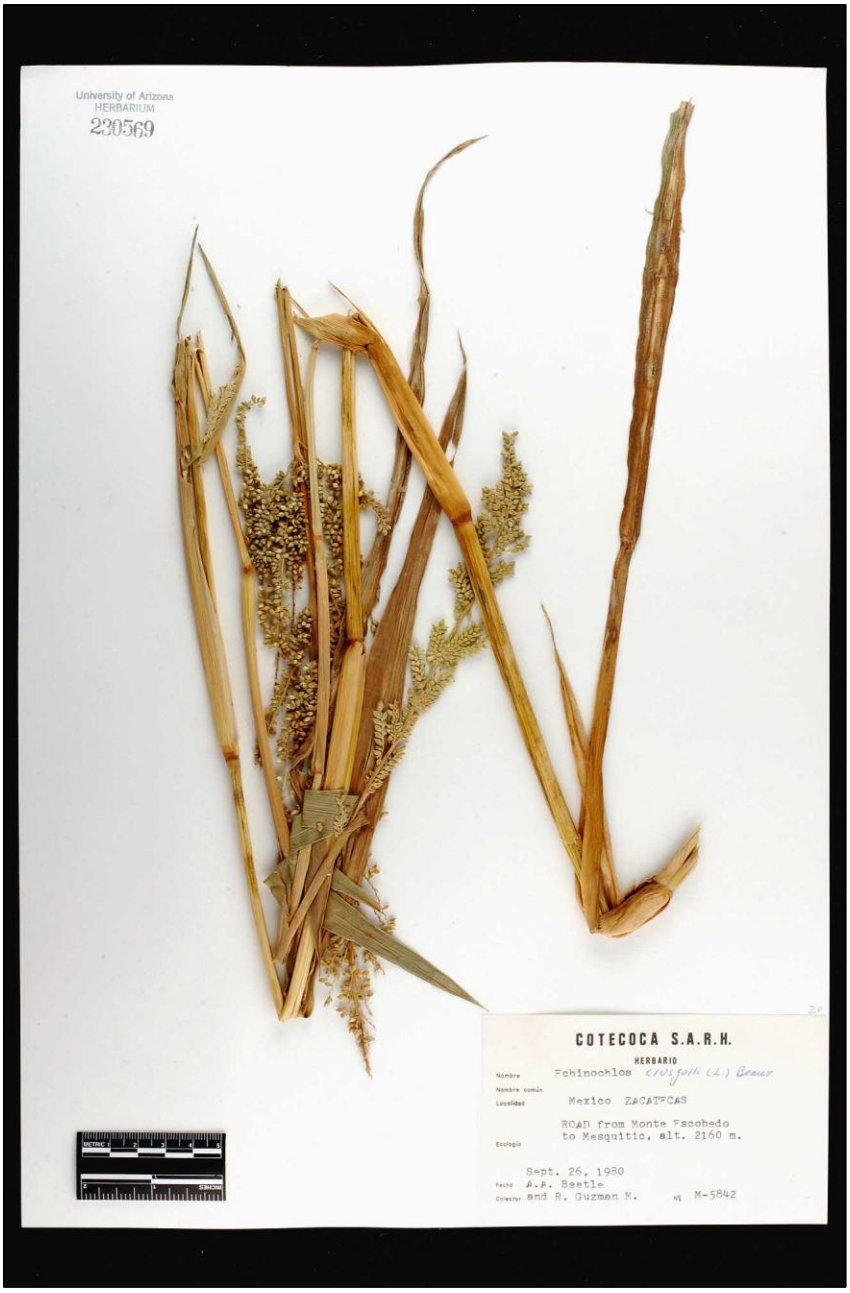


Figure F.3. Cockspur grass (*Echinochloa crus-galli*). Photo by University of Arizona. Public image from <http://www.conabio.gob.mx/invasoras> and used for educational purposes.


Figure F.4. Sand dropseed (*Sporobolus cryptandrus*). Photo by University of Arizona. Public image from <http://www.itis.gov> and used for educational purposes.


Figure F.5. Silky sophora (*Sophora nuttalliana*). Photo by U.S. National Herbarium, Smithsonian Institution. Public image from <http://www.itis.gov> and used for educational purposes.

# APPENDIX G: EXPANSION OF THE BREEDING RANGE OF THE BLUE-GRAY GNATCATCHER (*POLIOPTILA CAERULEA*) INTO WESTERN NEBRASKA

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## ABSTRACT

The Blue-gray Gnatcatcher (*Polioptila caerulea*) is a widespread species in the United States, and has been expanding its range northward. While eastern populations nest in deciduous trees, western populations nest in evergreen trees such as junipers (*Juniperus* spp.). In Nebraska, the species has been reported nesting only in the southeastern part of the state in mature riparian hardwood forest. We report the first nest of a Blue-gray Gnatcatcher found in western Nebraska. The nest was located in a red cedar (*Juniperus virginiana*). Given the affinity of the western populations for juniper-like habitat, the nesting birds in western Nebraska probably are from Wyoming or Colorado, reflecting a breeding range expansion of the western population.

The Blue-gray Gnatcatcher (*Polioptila caerulea*) is widespread in North America, extending northward as far as southeastern Minnesota and southern Maine in the East and southwestern Oregon and southwestern Wyoming in the West. As a forest-dweling species, it is not surprising that there is a breeding gap in the middle of the continent extending from northern Texas to Canada (Ellison, 1992; Fig. G.1).

The distribution of the Blue-gray Gnatcatcher has been changing in the last 40 years, with reported expansions of their summer range mainly towards the northeast into northern Wisconsin, northern Michigan, south central Ontario, southwestern Quebec, and much of New England (Kershner and Ellison 2012) and into the Dakotas (Faanes and Stewart 1982, Peterson 1995). More recently, western populations of the species have been expanding northward with records of occurrence in Utah (White et al. 1983), eastern Colorado (Sharpe et al. 2001), southwestern and south central Wyoming (Findholt 1983), south-central Washington (Wahl et al. 2005), and British Columbia (Davidson 2007); however, breeding records have only been obtained for Wyoming (Findholt 1983) and Washington (Wahl et al. 2005).

The Blue-gray Gnatcatcher has been reported as common in extreme eastern Nebraska (Zimmer 1917) and as a rare spring transient in western Nebraska (Brown and Brown 2001), although in recent years numbers of sightings have been increasing in the Nebraska Panhandle (Sharpe et al. 2001). The species was first recorded in western Nebraska during the summer in 1985 in the Platte Valley (Rosche 1994) and in 1993 at the Cedar Point Biological Station near Ogallala, when a female with a brood patch was captured near Lake Ogallala (Brown and Brown 2001). The species was only observed in a single location near the station between 2000 and 2006 (M. B. Brown, and J.

Faaborg, in litt.). In 2007 one individual was recorded several times in a different location near the station, and in 2009 at least three pairs were observed scattered throughout the station (J. Faaborg, in litt.). These records indicate that the species has become more abundant and widespread at this location.

There have been a few records of the species breeding in eastern and southeastern Nebraska since the 1960's (Ducey 1988), but the records have always been restricted to areas of mature riparian hardwood forest dominated by bur oak (*Quercus macrocarpa*), cottonwood (*Populus deltoides*), green ash (*Fraxinus pennsylvanica*), and hackberry (*Celtis occidentalis*) (Mollhoff 2001). A nest of a Blue-gray Gnatcatcher with a female found at the Cedar Point Biological Station (41°12'34.5"N, 101°38'54.4"W) on 14 June 2010 represents the first nest record of the species in western Nebraska. The nest was located in a red cedar (*Juniperus virginiana*) close to the dining lodge at about 3 m height (Fig. G.2). On 21 June one hatchling was found in the nest, but it appeared to be a brown-headed cowbird (*Molothrus ater*) nestling. The young bird was identified as a cowbird by its size and the color of its mouth lining. Blue-gray Gnatcatchers are one of the smallest hosts of the brown-headed cowbird (Root 1969, Friedmann et al. 1977). No young gnatcatchers were ever observed in the nest, similar to previous studies that have shown that parasitized gnatcatcher nests do not raise any of their own young (Goguen and Mathews 1996).

Eastern populations of Blue-gray Gnatcatchers nest in deciduous forested areas, often near rivers and lakes; western populations prefer dry and open pinyon-juniper woodland (Kershner and Ellison 2012). In Wyoming, where the species is known to have expanded from the southwest, nests of Blue-gray Gnatcatchers have been found only in

Utah juniper (*Juniperus osteosperma*) (Findholt 1983). Because the nest we found in western Nebraska was located in a red cedar, and virtually all local sightings of this species have been in cedars, we hypothesize that Blue-gray Gnatcatchers in this region are colonists from the population to the west. Expansion of the western population of the species has most likely occurred due to the predominant presence of junipers. Red cedars are expanding and changing the landscape cover in the Great Plains, converting remnant grassland habitats to red cedar savannas and woodlands (Gehring and Bragg 1992, Briggs et al. 2002, Horncastle et al. 2005). Recent studies have demonstrated shifts in avian communities associated with red cedar encroachment. Open woodland species of birds, such as the Blue-gray Gnatcatcher, are predicted to increase with red cedar expansion (Coppedge et al. 2001, Chapman et al. 2004, Coppedge et al. 2004). The increase of red cedars along the hills bordering the North Platte River possibly provides a corridor for movement of birds from the west.

## ACKNOWLEDGEMENTS

I would like to thank M. B. Brown for providing valuable information regarding historical records of the species at the Cedar Point Biological Station, and L. E. Ramírez for taking the picture of the nest and providing assistance with the translation of the abstract.

## LITERATURE CITED

Briggs, J. M., G. A. Hoch, and L. C. Johnson. 2002. Assessing the rate, mechanisms, and consequences of the conversion of tallgrass prairie to *Juniperus virginiana* forest. Ecosystems 5:578-586.

Brown, C. R., and M. B. Brown. 2001. Birds of the Cedar Point Biological Station. Occasional Papers of the Cedar Point Biological Station 1:1-36.

Chapman, R. N., D. M. Engle, R. E. Masters, and D. M. Leslie, Jr. 2004. Tree invasion constrains the influence of herbaceous structure in grassland bird habitats. Ecoscience 11:55-63.

Coppedge, B. R., D. M. Engle, R. E. Masters, and M. S. Gregory. 2001. Avian response to landscape change in fragmented southern Great Plains grasslands. Ecological Applications 11:47-59.

Coppedge, B. R., D. M. Engle, R. E. Masters, and M. S. Gregory. 2004. Predicting juniper encroachment and CRP effects on avian community dynamics in southern mixed-grass prairie. Biological Conservation 115:431-441.

Davidson, G. S. 2007. Current status of the blue-gray gnatcatcher in British Columbia. Wildlife Afield 4:38-42.

Ducey, J. E. 1988. Nebraska Birds, Breeding Status and Distribution Simmons-Boardman Books, Omaha, Nebraska.

Faanes, C. A., and R. E. Stewart. 1982. Revised checklist of North Dakota birds. The Prairie Naturalist 14:81-92.

Findholt, S. L. 1983. First nest records for the plain titmouse and blue-gray gnatcatcher in Wyoming. Great Basin Naturalist 43:747-748.

Friedmann, H., L. F. Kiff, and S. I. Rothstein. 1977. A further contribution to knowledge of the host relations of the parasitic cowbirds. Smithsonian Contributions to Zoology 235:1-75.

Gehring, J. L., and T. B. Bragg. 1992. Changes in prairie vegetation under eastern red cedar (*Juniperus virginianan* L.) in an eastern Nebraska bluestem prairie. American Midland Naturalist 128:209-217.

Goguen, C. B., and N. E. Mathews. 1996. Nest desertion by blue-gray gnatcatchers in association with brown-headed cowbird parasitism. Animal Behaviour 52:613-619.

Horncastle, V. J., E. C. Hellgren, P. M. Mayer, A. C. Ganguli, D. M. Engle, and D. M. Leslie Jr. 2005. Implications of invasion by *Juniperus virginiana* on small mammals in the southern Great Plains. Journal of Mammalogy 86:1144-1155.

Kershner, E. L., and W. G. Ellison. 2012. Blue-gray Gnatcatcher (*Polioptila caerulea*), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America Online: http://bna.birds.cornell.edu.bnaproxy.birds.cornell.edu/bna/species/023.

Mollhoff, W. J. 2001. The Nebraska Breeding Bird Atlas: 1984-1989 Nebraska Game and Parks Commission, Lincoln, Nebraska.

Peterson, R. A. 1995. The South Dakota Breeding Bird Atlas. Northern State University, Aberdeen, South Dakota.

Root, R. B. 1969. The behavior and reproductive success of the blue-gray gnatcatcher. The Condor 71:16-31.

Rosche, R. C. 1994. Birds of the Lake McConaughy Area and the North Platte River Valley, Nebraska. Richard C. Rosche, Chadron, Nebraska.

Sharpe, R. S., W. R. Silcock, and J. G. Jorgensen. 2001. Birds of Nebraska: Their Distribution & Temporal Occurrence. University of Nebraska Press, Lincoln, Nebraska and London, United Kingdom.

Wahl, T. R., B. Tweit, and S. G. Mlodinow. 2005. Birds of Washington: status and distribution. Oregon State University Press, Corvallis, Oregon.

White, C. M., H. H. Frost, D. L. Shirley, G. M. Webb, and R. D. Porter. 1983. Bird distributional and breeding records for southeastern Idaho, Utah, and adjacent regions. Great Basin Naturalist 43:717-727.

Zimmer, J. T. 1917. A few interesting spring migration records from Lincoln, Nebraska. The Wilson Bulletin 29:193-194.

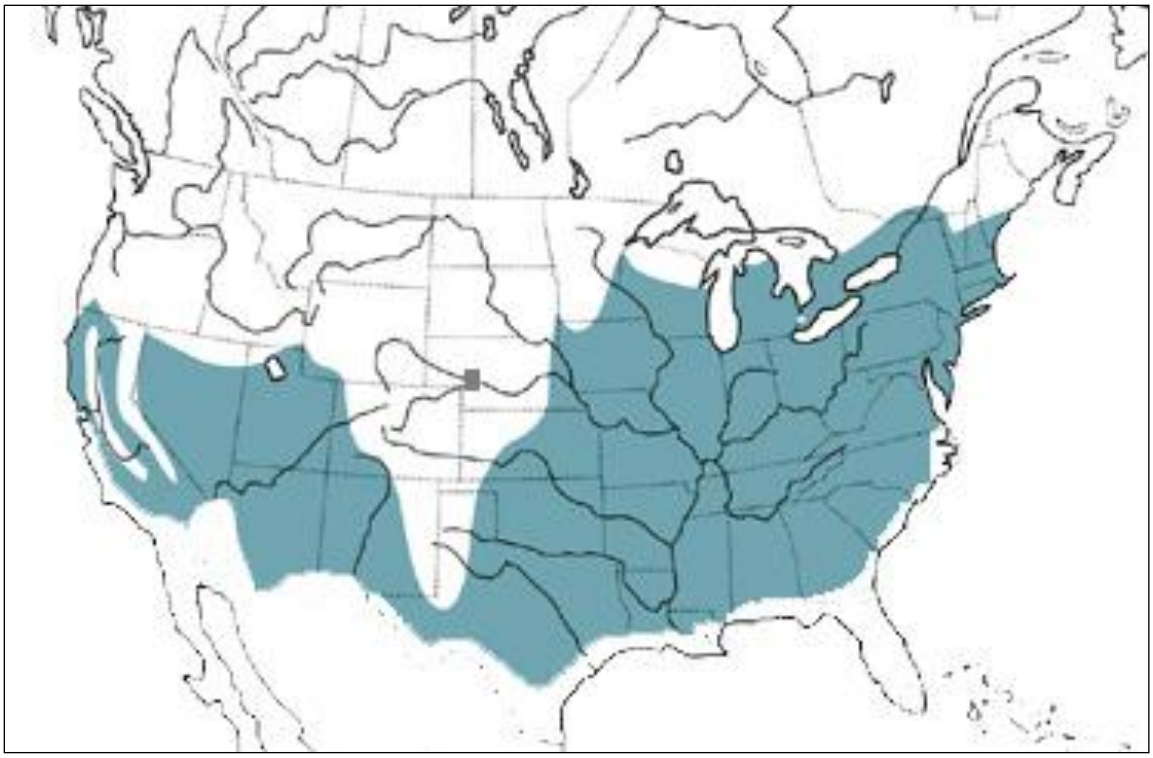

Figure G.1. Breeding distribution of the Blue-gray Gnatcatcher (*Polioptila caerulea*) with rectangle marking where the nest was located in western Nebraska (Map courtesy of Birds of North America Online: http:/bna.birds.cornell.edu/bna, maintained by the Cornell Lab of Ornithology).

Figure G.2. Blue-gray Gnatcatcher (*Polioptila caerulea*) feeding a Brown-headed Cowbird (*Molothrus ater*) nestling near Ogallala, western Nebraska, 21 June 2010 © Luis E. Ramírez.