ASSESSING LEVELS OF TOTAL MERCURY IN THE ENDANGERED WHOOPING CRANE (*Grus americana)*

By Paige E. Smith

Whooping cranes (*Grus americana*) are one of the most charismatic and endangered birds in North America. Currently, there are 594 wild individuals found in four populations, one of which is the eastern migratory population (EMP) that breeds in central Wisconsin. Unfortunately, reproductive success in the EMP has been close to 0%.

We hypothesized that mercury, an anthropogenic environmental contaminant, hinders the cranes' ability to rear offspring to independence. This hypothesis stems from the fact that (1) mercury has been measured in Wisconsin breeding grounds and (2) when methylated, mercury can alter a wide range of behaviors including parental care, foraging, and reproductive success. To test this, we measured total mercury in three whooping crane populations: Aransas-Wood Buffalo (AWBP), the EMP, and captive birds from the International Crane Foundation (ICF). This allowed us to compare birds of known successful reproduction (ICFP), birds of presumed successful reproduction (AWBP), and birds of known low reproduction (EMP). If our hypothesis is true, we predicted that mercury would be higher in the EMP than in the AWB and ICF.

Results show elevated levels of mercury in the EMP as compared to the AWBP and ICFP. Our study is novel in that whole blood samples were measured for a baseline in multiple populations of endangered whooping cranes. The results show low levels of mercury in all WHCR populations; however, there is no agreement on what a safe threshold is for humans let alone other mammals or birds. More research is needed to examine the sub lethal effects contaminants such as mercury has on *G. americana.*

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by

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To my family and advisor, I dedicate this thesis. To my advisor, mentor, and friend, Dr. Misty McPhee, for her shining example of what it truly means to be a dedicated researcher, professor, and animal advocate. To my sister, Haley whose friendship and love has guided me in ways only a sister can. A special feeling of gratitude to my loving parents, Ronald and Kelli whose words of encouragement and unwavering support will forever be treasured.

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Chapter I:
Whooping Cranes

History of *Grus americana*

Worldwide there are currently 15 species of crane; seven species are listed as vulnerable (*Anthropoides paradiseus, Antigone, Antigone vipio, Balearica pavonina, Bugeranus carunculatus, Grus monacha,* and *G. nigricollis*), three species are listed as endangered (*Balearica regulorum,G. americana, and G. japonensis*), and one is listed as critically endangered (*Leucogeranus leucogeranus*) (IUCN, 2016). Cranes are generally split into two groups, typical and crowned. Typical cranes are separated from crowned cranes due to their coiled trachea, tighter body plumage and ability to withstand colder environments (Brodkorb, 1967). Typical cranes first appear in the fossil record in the Miocene period, dating back 5-24 million years ago (Miene, 1996). There are two species of typical cranes that inhabit North America, the sandhill crane (*G. canadensis*) and whooping crane (*G. americana*) with whooping cranes being one of the most endangered birds in North America. In the late 1800s, there were more than 1,000 whooping cranes surviving in likely multiple populations (Allan, 1952). By 1941, an estimated 15 birds remained in the wild and one pair remained in captivity (USFWS, 1997). Whooping cranes (WHCR) are classified within the family *Gruidae*.

Population declines of the whooping crane were caused by destruction of nesting habitat for land settlement and agriculture during westward expansion (Erickson & Derrickson, 1981; Glenn et al., 1999). Crane numbers also decreased due to sport and subsistence hunting (Allan, 1952). In 1967, the United States Fish and Wildlife Service (USFWS) listed whooping cranes as threatened with extinction and upgraded them to endangered in 1970. In 1978, Canada designated the whooping crane as endangered by the Committee on the Status of Endangered Wildlife in Canada (CWS and USFWS, 2007). Per the Endangered Species Act, critical habitat in the United States was designated in 1978 (USFWS, 1994). In 2003, critical habitat in Canada was designated with the final publication of the Species at Risk Act (SARA), (CWS and USFWS, 2007).

The two migratory whooping crane populations, the Aransas-Wood Buffalo population (AWBP) and the reintroduced eastern migratory population (EMP), share similar habitat requirements for both breeding and wintering territories. Breeding territories have specific requirements including access to shallow wetlands and a large territory separated from other breeding pairs (Erickson & Derrickson, 1981). Whooping cranes prefer to roost in aquatic wetlands or salt marshes during both winter and summer seasons (Gomez, 1991). These habitats are very susceptible to alteration or degradation through anthropogenic activities such as land settlement and agricultural development (Erickson & Derrickson, 1981).

Whooping Crane Conservation Efforts

There are only 594 individuals remaining in four wild populations: the migratory Aransas-Wood Buffalo population (the only self-sustaining population, N = 431), the reintroduced non-migratory population in Louisiana (N = 49), the reintroduced non-migratory population in central Florida (N = 15), and the reintroduced EMP, which is released into central Wisconsin (N = 100) (WCEP, 2018, ICF, 2018). Protecting the species from extinction and delisting the whooping cranes are the main objectives for managers in the United States and Canada. Whooping cranes face several threats as a species: predation, shooting, habitat loss, stochastic environmental events, collisions with anthropogenic objects, and loss of genetic diversity (CWS and USFWS, 2006). The third revision of the International Recovery Plan (IRP) (CWS and USFWS, 2007) outlines criteria for meeting these objectives.

The first criterion states that a minimum of 40 productive pairs must be present in the AWBP, plus 25 productive pairs in two other populations. To consider downlisting the species from endangered to threatened, all three populations would need to be self-sustaining for a minimum of a decade (USFWS and USFWS, 2007). If this is not feasible, two alternatives have been proposed. First, the AWBP must reach 100 productive pairs and a second population must remain above 30 productive pairs. Again, to be considered for downlisting the species from endangered to threatened; both populations would need to be self-sustaining for a minimum of a decade. The other alternative

states that, if a second population is unable to remain self-sustaining, the AWBP must reach 250 productive pairs and 1,000 individuals for down listing to be considered. In 1985, Canadian and U.S. officials approved the *Memorandum of Understanding on Conservation of Whooping Cranes* (MOU) that recognized 1,000 individuals as the goal for the species.

The second criterion is to maintain a genetically stable captive population. Ensuring genetic variability is defined as maintaining 21 productive pairs in captivity (CWS and USFWS, 2007). To better understand how to introduce a new population into the wild there were several attempts made to establish supplemental populations, all of which were deemed experimental and nonessential (USFWS, 1997). These populations include the Grays Lake population, the Florida nonmigratory population, and the eastern migratory population.

Aransas Wood-Buffalo Population. The Aransas Wood-Buffalo population is a relictual population, which is the remnant of the original wild whooping crane population. To maximize the population size in the 1960s, eggs were collected from pairs' nests. Eggs were hatched and the chicks were reared in captivity by aviculturists. This process led to the original captive flock and the capability for captive propagation and reintroductions. Presently, the AWBP winters on the Gulf Coast of Texas, USA, at the Aransas National Wildlife Refuge (ANWR). The breeding grounds for AWBP are in Wood Buffalo National Park, Northwest Territories, Canada. The AWBP breeding grounds are surrounded by

headwaters of four different rivers and interspersed with shallow wetlands. The cranes nest in potholes that are dominated by aquatic plants such as *Typha*, *Carex,* and *Chara* (Timoney, 1999). The wintering ground habitats are characterized as estuarine marshes, shallow bays, and tidal flats. The marshes are dominated by salt grasses (*Distichlis spicata*), saltwort (*Batis maritima*), smooth cordgrass (*Spartina alterniflora*), and sea ox-eye (*Borrichia frutescens*) (Hassett, 2006). The interior of the refuge is sandy and dominated by Gulf cordgrass live oak (*Quercus virginiana*), redbay (*Persea borbonia*), and bluestem (*Andropogon* spp.) (Hassett, 2006).

Grays Lake Population. The Grays Lake population (GLP) was an experimental group of whooping cranes that was introduced at Grays Lake NWR in Idaho (USFWS, 1997). The GLP migrated between Idaho and New Mexico and was designated as a non-essential migratory population. This population was established between 1975 and 1989 by translocating 216 whooping crane eggs retrieved from AWBP nests and introducing 73 eggs from Patuxent Wildlife Research Center (PWRC) into Rocky Mountain greater sandhill crane nests at Grays Lake NWR in Idaho (Hartup, in press). The sandhill cranes would then foster parent the whooping crane chicks, teaching them the migratory route. The GLP reached its maximum number of 31 individuals and was extirpated in 2002 (CWS & USFWS, 2007). This population was not successful for a variety of reasons; most importantly, that the whooping crane chicks imprinted on sandhill cranes and breeding between released whooping cranes never occurred

(USFWS, 1997). With this failure, researchers noted that sandhill cranes foster-parenting WHCR chicks would not create a self-sustaining population of whooping cranes (USFWS, 1997).

Florida Non-Migratory Population. Following the WHCR Recovery Plan, a population of birds was introduced in Kissimmee, Florida beginning in 1993. This population was intended to be non-migratory and make use of offspring from captive breeding efforts. To prevent imprinting on sandhill cranes, the chicks were raised in captivity either by foster WHCRs or by aviculturists dressed in crane costumes (Folk et al., 2008). Once fledged, the birds were soft released in Florida. Between 1993 and 2004, 289 birds were released. By 2002, a pair successfully hatched and fledged the first wild WHCR chick from a reintroduced population and the first chick to hatch in the United States since 1939 (Folk et al., 2008). Unfortunately, due to high predation, drought, and habitat loss, reproductive rates were slow and mortality rates were very high. This led to the reintroduction efforts in Florida ending in 2008.

Eastern Migratory Population. To assist in the protection of the species, the Whooping Crane Eastern Partnership (WCEP) was established in 1999 and was initially comprised of nine state and federal agencies and nonprofit organizations (Urbanek, et al., 2005). WCEP's mission was to establish a second self-sustaining migratory population apart from the AWBP. A second population would serve as a safeguard for the species from possible extinction by factors such as disease or environmental catastrophe. Under the auspices of

WCEP, the EMP's first captive-reared whooping cranes were released in 2001 into the Necedah National Wildlife Refuge (NWR), Juneau County, Wisconsin, USA. Based on a thorough habitat analysis of various Midwestern locations (Canon, 1999), WCEP deemed Necedah NWR to have the necessary habitat for the whooping crane's breeding grounds. As of 2018, the birds are wintering in Illinois, Indiana, Kentucky, Tennessee, Alabama, Georgia and Florida, USA (WCEP, 2018). During migration, the birds roost in shallow open wetlands and forage in agriculture farmland (Channell & Lomolino, 2000). The EMP birds summer and breed in western Wisconsin. Often in both the AWBP and EMP, the birds will venture to neighboring agricultural land in search of food. This foraging behavior occurs in the AWBP during migration and wintering, and in the EMP throughout the year. While Necedah NWR and other central and eastern Wisconsin sites are the summer grounds for the birds, this area is on the periphery of the core whooping crane geographical range and distribution. Thus, this geographic region may not be optimal habitat for the cranes. Currently, the EMP has a population of 110 birds (WCEP, 2018).

In the Necedah NWR, the birds typically use shallow waters and emergent wetland vegetation that borders impoundments. The birds also use palustrine and upland scrub-shrub areas during the day for foraging and exploratory activities. During early and midsummer, the EMP birds can be seen foraging on blueberries (*Cyanococcus*) and sarsaparilla (*Smilax*). During pool draw-downs,

ephemeral foraging habitat is created, giving the cranes access to trapped fish and aquatic prey (Hassett, 2006).

The formation of the EMP was accomplished through captive propagation and fledgling release. The EMP birds were raised in captivity by aviculturists dressed in crane costumes, similar to the birds raised for the Florida non-migratory population. Once the birds were released, they were trained to follow an ultralight aircraft on the migration route from Wisconsin to Chassahowitzka NWR in central Florida (Urbanek et al., 2010, Hartup, in press, Operation Migration, 2018).

Whooping Crane Reproduction

Since the beginning of releases in 2001, survivorship of juveniles and adults in the EMP has been 88% for unpaired birds and 99% for paired birds (Harrell & Bidwell 2016). Despite this overwhelming success, the birds' reproductive success has been poor as the chick survival rate of the EMP is estimated at <5% (B. Hartup, personal communication, 2018). Every year birds have been released into the EMP from various captive breeding centers but until reproductive success increases, the EMP will not be able to maintain a self-sustaining population of whooping cranes.

Birds released in the EMP were raised at PWRC or ICF either by a human in a costume or by a foster WHCR pair. Several new captive breeding centers

established whooping crane programs in 2018, with unpredictable impacts on numbers of chicks for release.

Many hypotheses have been proposed to explain the low reproductive success of the EMP WHCRs. One hypothesis suggests that the captive rearing of the birds contributes to their low reproductive success (Sadowski et al., 2018). The captive selection hypothesis could influence low reproductive success and failure to fledge. Along with this, a second hypothesis proposes that selection pressures on captive-reared cranes are relaxed in comparison to wild-born cranes, which may lead to ineffective predator response behaviors of adult cranes (Stewart et al., in preparation). A different hypothesis suggests that black fly disturbance during incubation leads to nest abandonment and eventual failure (King et al., 2015). A fourth hypothesis is that exposure to contaminants such as mercury can impair the nervous system (Shore et al., 2011) as well as behavior, physiology, (Frederick and Jayasena, 2011) and perhaps more importantly reproductive success (Daso et al., 2015). Of the many hypotheses proposed, exposure to contaminants has been one of the least researched.

Chapter II:
MERCURY AND WILDLIFE

Many hypotheses have been proposed to explain the low reproductive success of the EMP birds however; contaminants are one of the least researched. The environmental contaminant mercury and its toxic effects could affect reproductive success in the EMP.

Global Mercury

Mercury is a ubiquitous, naturally occurring element that has been used by a wide range of industries for a variety of purposes, such as in electrical appliances, medical devices, and paper manufacturing. This use has led to high levels of environmental release. Unlike many other contaminants, mercury is highly persistent and will remain in the environment forever, as it does not degrade (Rattner et al., 2011). The change in human activities has increased the amount of mercury cycling through the environment three to five times since pre-industrialization (Selin, 2009). Globally, concerns exist around the toxic properties of mercury and its ability to contaminate water sources through runoff from industrial sites and abandoned mines, and atmospheric deposition (Fleming et al., 2006).

Atmospheric deposition of mercury occurs when emitted into the atmosphere in its elemental form and is able to achieve global distribution before

oxidizing into a form that deposits into an ecosystem. There are several areas of uncertainty in the biogeochemical cycle of mercury such as the oxidation processes in the atmosphere, land-atmosphere cycling, and the process of methylation in the oceans (Selin, 2009, Lin & Pehkonen, 1999). Researchers studying the biogeochemical cycle of mercury have developed multiple hypotheses to explain some aspects of global mercury distribution, but have no definitive explanation for these areas (Selin, 2009, Fleming et al., 2006, Lin & Pehkonen, 1999).

Due to the global pollution potential of mercury, international groups have formed to manage and assess mercury threat levels. Mercury is one of many contaminants that are listed under the Heavy Metals Protocol to the Convention on Long-range Transboundary Air Pollution (Pacyna et al., 2010). Mercury is also monitored under the United Nations Environment Programme (UNEP). A major component in the UNEP initiative has been to obtain accurate information on current mercury atmospheric emissions and trends.

According to the United States Environmental Protection Agency, there are several anthropogenic causes to atmospheric mercury release such as artisanal and small-scale gold mining, coal combustion, and non-ferrous metal and cement production (United Nations Environment Programme, Global Mercury Assessment, 2013). Globally, total annual mercury emissions from both natural and anthropogenic sources are estimated to range from 5,000-8,000 metric tons. In Texas, annual mercury emissions are estimated at just over 6

metric tons, with Texas having the highest amount of mercury emitted in the United States each year, at 12.32% of total U.S. mercury (Bolate, 2017). In Wisconsin, annual mercury emissions are estimated at approximately 0.6 metric tons, with Wisconsin having a low amount of mercury emitted in the United States each year, at 1.23% of total U.S. mercury (Bolate, 2017). The differences in mercury deposition can be explained by the differences in anthropogenic activities in the two regions. Mercury trends are monitored in the United States at sites known as Mercury Deposition Network sites. Wisconsin has multiple MDN sites; however, they are focused on northern Wisconsin, whereas Necedah and Baraboo are located in the central western part of the state.

Biogeochemical Cycling

Mercury is released from either a natural or an anthropogenic source from terrestrial or aquatic surfaces in its elemental form, Hg^0, which can remain in the atmosphere from six months to a year. Anthropogenic sources can also emit mercury in two other forms, divalent Hg^{2+} and particulate matter associated with mercury, Hg^P (Schroeder & Munthe, 1998). Neither of these forms of mercury remain in the atmosphere for long, only days to weeks. Hg^{2+} and Hg^P are soluble in water and therefore are the predominant forms of mercury deposited in ecosystems via wet and dry deposition (Selin, 2009). Wet deposition is the process by which mercury is removed from the atmosphere during precipitation. Dry deposition is the settling or uptake of mercury to the earth's surface without

precipitation. Mercury is released from terrestrial and aquatic systems through volatilization.

Wet and dry deposition in aquatic and terrestrial systems is predominantly Hg^{2+}. From this state, mercury either is typically volatilized to Hg^0 in the atmosphere or can convert to its more toxic form, MeHg. The final deposition of mercury is when mercury is embedded in deep-ocean sediments, settling through the water column (Selin, 2009). This can take approximately 3,000 years from the beginning of the cycle in the atmosphere to the settling in ocean sediments and the end of the cycle (Selin, 2009).

Mercury deposition can wreak havoc on freshwater ecosystems. This is due in part to the limited dissolved oxygen concentrations and abundant organic matter that characterize wetlands, creating favorable conditions for bacteria to convert mercury to methylmercury.

Mercury Methylation

Sediments serve as the main reservoir of Hg in freshwater systems (Ullrich et al., 2001). Abiotic methylation of mercury may occur in compounds such as methyl iodide, dimethyl sulfide, and dissolved organic matter such as fulvic and humic acids (Celo et al., 2006). The methylation of mercury in an abiotic environment is influenced by pH, temperature, and the presence of the donor complexes required in the pathways such as chloride. Donor complexes provide a mechanism for transportation of ions for methylation (Gu et al., 2011).

Sediments can act as both a sink and a potential source of elemental Hg and it can remain in the aquatic sediments for many years (Ullrich et al., 2001). In freshwater and estuarine waters, organic colloids comprise the majority proportion of Hg, with 90% being complexed by organic matter (Ullrich et al., 2001).

Methylation of mercury can occur through sulfate-reducing bacteria, *Desulfobulbus propionicus*, in various anoxic sediments when a carbonyl group is added to Hg^0 (Fleming et al., 2006). The mechanisms of mercury methylation via sulfate-reducing bacteria are not completely known. In oxic conditions, the process of methylation does not occur. Iron-reducing bacteria in the genus *Geobacter* have also been shown to methylate mercury at environmentally significant rates similar to the rates of sulfate-reducing bacteria (Fleming et al., 2006).

Effects on Wildlife

Methylmercury (MeHg) is a neurotoxicant, an endocrine disruptor, and a teratogen (Shore et al., 2011). As previously stated, iron-reducing and sulfur-reducing bacteria in the environment metabolize the mercury to form either MeHg or inorganic mercury (Hg), which differ considerably in dietary absorption, tissue distribution, toxicity and exposure to wildlife (Rattner et al., 2011). The biological half-life of MeHg in blood is 39 to 70 days depending on the individual size of the animal and MeHg concentrations (Rice et al., 2014). Multiple studies have linked

an increase in MeHg concentrations found in piscivorous birds and mammals with reduced reproductive success, behavioral changes, and kinetic impairment (Ward et al., 2010, Scheulhammer et al., 2007, Wolfe et al., 1998). MeHg represents 95% of the total mercury found in birds (Celo et al., 2006).

The animal and human health concern with this metal is that it biomagnifies and bioaccumulates as it moves up a food chain, meaning predators can be exposed to high dietary concentrations of MeHg (Shore et al., 2011). In freshwater systems, an increase in algae blooms can reduce MeHg concentrations in the water column (Pickhardt et al., 2002) because it can accumulate in algae. Once Hg is in the algae, it can be consumed and biomagnified throughout the food web (Hunter et al., 2003). Primary and secondary consumers ingest zooplankton that feed on the algae. Due to its protein-binding properties, aquatic biota can readily accumulate high levels of MeHg (Ullrich et al., 2001). MeHg has a high bioaccumulation factor of about 10 million, leading to toxic levels at higher trophic levels (Driscoll et al., 2007). Bioaccumulation results in high concentrations of mercury stored in the liver, kidney, and muscle; it is also excreted in hair and feathers providing an excellent biomarker to track this metal within a bird (Shore et al., 2011). Due to the bioaccumulation of Hg^{2+} and MeHg, both forms can remain present in a given ecosystem for hundreds of years, simply moving up the food chain and being consumed during predation and foraging. Mercury is introduced to birds typically through consumption of prey items. Ackerman et al., 2016 suggests sampling

from tissues that are easily translated into a tissue type that has a toxicity benchmark and is directly relevant to bird reproduction such as adult blood, eggs, and chick down feathers. Total mercury (THg) concentrations measured in whole blood are used to estimate MeHg concentrations found in tissues as 70-95% of THg is MeHg (Mortensen et al., 2014). In avian organs such as the liver, MeHg is transformed into a less toxic form, inorganic mercury. Inorganic mercury can be stored in the internal organs in this form at high concentrations before being transferred to feathers (Ofukany, 2012). During the period of feather growth, measuring mercury in feathers can serve as a proxy for blood levels in migratory species from which blood samples are difficult to obtain. Species such as the common loon (*Gavia immer*), common tern (*Sterna hirundo*), and double-crested cormorant (*Phalacrocorax auritus*) accumulate mercury in summer-grown feathers and may therefore transport mercury from northern breeding sites to the wintering grounds (Ofukany, 2012).

Review of wildlife mercury studies. Experimental and comparative work that examines the effects of MeHg on birds is plentiful. Birds store contaminants in endogenous energy reserves such as lipids and can be transferred to eggs (Ofukany, 2012). Avian reproduction is sensitive to mercury toxicity because it affects bird health, behavior, and productivity (Scheuhammer et al., 2007, Ackerman et al., 2016). In the 1970s, Heinz found that MeHg affected egg weight, “soundness” of eggs, hatching success, chick mortality within first week of life, and percent weight gain in mallard ducks (*Anas*

platyrhynchos) (1974) as well as affecting reproductive success and behavior across generations (1979). Bouton et al. (1999) documented that high levels of MeHg negatively affected activity levels, habitat use, and hunting ability in great egret chicks (*Ardea albus*). Female tree swallows (*Tachycineta bicolor*) living in a highly contaminated area along the Shenandoah River, Virginia, USA produced fewer fledglings than females that lived along a less contaminated stretch of the same river (Brasso and Cristol 2008). In common loons (*Gavia immer*), high adult blood mercury levels led to decreased time that both males and females spent incubating eggs as well as decreased time spent in high energy behaviors while brooding their young (Evers et al., 2008). Frederick and Jayasena (2011) experimentally tested the effects of MeHg on courtship, pairing behavior, and breeding success in captive white ibises; dosed groups had significantly more unproductive nests than non-dosed group (Frederick and Jayasena, 2011).

There have been a limited number of studies done on typical cranes concerning mercury as a contaminant and the threshold levels for these birds. Cranes do forage in aquatic wetlands and agricultural farmlands, both of which are susceptible to environmental contaminants (Cheng et al., 2011). One study measured total Hg and MeHg in the prey species of red-crowned cranes (*G. japonensis*) in the Zhalong Wetland in northeastern China (Luo et al., 2014). Researchers also examined feathers and feces of the red-crowned cranes and found elevated levels of THg), but MeHg was below the detection limit. Red-crowned cranes were concluded to have low levels of THg and negligible levels

of MeHg. There was no threshold limit to MeHg stated by researchers and thus is still undetermined.

To this author's knowledge, there have been no behavioral studies on whooping cranes exposed to a toxic contaminant such as mercury. Mercury can be passed down from the avian mother to her eggs in the form of MeHg. In other bird species, the presence of MeHg in eggs can result in embryonic mortality, lower hatchability, chick malformations, and a decrease in chick survivorship (Grajewska et al., 2015). There have been no studies regarding the effects of MeHg and whooping crane chicks. The effects of MeHg concentrations vary between different bird species. In most bird species, mercury is produced and eliminated by transporting MeHg from blood and organs to the growing feathers. MeHg has high binding affinity for free thiol groups, which are plentiful in the keratin of feathers (Rouse & Van Dyke, 2010). The mercury load in feathers is about 60-70% of the total mercury found in the bird. These feathers will grow until the next molt, which will be shed during the molt thus removing mercury from the crane (Teraoka et al., 2015). In songbirds, researchers measured the level of THg in blood. They found blood THg levels are not indicative of contamination levels as a large portion of the ingested mercury was sequestered to the feathers (Condon & Cristol, 2009).

Several factors may affect the impact of MeHg on traits such as foraging, body size, life span, and molt strategy (Walsh, 1990). These factors impact the effect of MeHg differently in every species of wetland bird, in *G. americana* MeHg

and its effects on chicks has not been well studied. Regarding the common loon, a study was conducted measuring pH and mercury levels in Iron, Oneida and Vilas Counties, Wisconsin, and Nova Scotia, and New Brunswick, Canadian lakes (Burgess & Meyer, 2008). Researchers found that Wisconsin had significantly higher mean lake pH versus the Canadian lakes and that the blood mercury levels of adult and juvenile birds decreased with a decrease in lake pH. They concluded that an increase in MeHg exposure led to a decrease in loon reproductive success (Burgess & Meyer, 2008).

The literature often uses birds and fish that are listed as least concern as bioindicators. These organisms can be monitored regularly for mercury levels, which provides information on the mercury status in wetlands (Zillioux et al., 1993). The literature is also rife with sources and studies examining the toxicity of mercury on wetland species using captive species. The issue with these studies is that the typical bird species being examined in captivity are not strictly piscivorous and would likely have a different sensitivity to MeHg than piscivorous birds; this could lead to inaccuracies in predicting the sensitivity of piscivorous birds to mercury (Evers et al., 2003). Omnivorous birds such as whooping cranes could experience behavioral or physiological change due to mercury exposure. This is especially problematic for small chicks or colts.

As the EMP reproductive success continues to remain low, researchers continue to examine the cause for the low success. Contaminants such as mercury have the toxicity to affect both the adult WHCRs and chicks in a

multitude of ways. The EMP requires human intervention as this population is one of two wild migratory populations of WHCRs. This population was established to protect the species from extinction and allow for delisting (CWS and USFWS, 2006).

Chapter III:
ASSESSING LEVELS OF TOTAL MERCURY IN THE ENDANGERED WHOOPING CRANE (*Grus americana*)

Introduction

In the late 1800s, there were more than 1,000 whooping cranes (*G. americana*) (Allen, 1952). By 1941, an estimated 15 birds remained in the wild and one pair remained in captivity (USFWS, 1997). Population declines of whooping cranes were caused by sport and subsistence hunting, and by destruction of nesting habitat for land settlements and agriculture during westward expansion (Erickson & Derrickson, 1981, Glenn et al., 1999, Allen, 1952). There are only 594 individuals remaining in four wild populations: the migratory Aransas-Wood Buffalo population (the only self-sustaining population, N = 431), the reintroduced non-migratory population in Louisiana (N = 49), the reintroduced non-migratory population in central Florida (N = 14) and the reintroduced eastern migratory population that is released into central Wisconsin (N = 100) (WCEP, 2018; ICF, 2018). Protecting the species from extinction and delisting the whooping cranes are the main objectives for managers in the United States and Canada. *Grus americana* faces several threats today as a species: predation, shooting, habitat loss, stochastic environmental events, collisions with anthropogenic objects, and loss of genetic diversity (CWS and USFWS, 2006). Whooping cranes prefer to roost in aquatic wetlands during both winter and summer seasons (Gomez, 1991). Wetland habitats are susceptible to alteration

or degradation through various anthropogenic activities such as land settlement and agricultural development (Erickson & Derrickson, 1981).

The Aransas-Wood Buffalo population (AWBP) winters on the Gulf Coast of Texas, USA, at the Aransas National Wildlife Refuge (NWR). The summering ground habitats are characterized as estuarine marshes, shallow bays, and tidal flats. Based on a thorough habitat analysis of various Midwestern locations (Canon, 1999) the Whooping Crane Eastern Partnership deemed Necedah NWR to have the necessary habitat for the eastern migratory population (EMP) breeding grounds. The eastern migratory population (EMP) birds roost in shallow open wetlands and forage in agriculture farmland (Hassett, 2006). While Necedah NWR and other central and eastern Wisconsin sites are the summer grounds for the birds, this area is on the periphery of the core whooping crane geographical range and distribution.

Since the beginning of releases in 2001, annual survivorship of juveniles and adults in the EMP has been 88% for unpaired birds and 99% for paired birds (Harrell & Bidwell 2016). Despite this, the birds' reproductive success has been low. One hypothesis to explain low reproductive success of the EMP is that exposure to contaminants such as mercury can impair the nervous system (Shore et al., 2011) as well as behavior, physiology, (Frederick and Jayasena, 2011) and perhaps more importantly reproductive success (Daso et al., 2015).

Mercury is a naturally occurring element that has been used by a wide range of industries leading to high levels of environmental release. Unlike many

other contaminants, mercury is highly persistent and will remain in the environment forever (Rattner et al., 2011). MeHg is a neurotoxicant, an endocrine disruptor, and a teratogen (Shore et al., 2011). The animal and human health concern with this metal is that it biomagnifies as it moves up a food chain, meaning predators could be exposed to high dietary concentrations of MeHg (Shore et al. 2011). This leads to high concentrations of mercury stored in the liver, kidney, and muscle; it is also excreted in hair and feathers providing an excellent biomarker to track this metal within a bird (Shore et al., 2011). Ackerman et al. (2016) suggested sampling from tissues that are easily translated into a tissue type that has a toxicity benchmark and is directly relevant to bird reproduction such as adult blood, eggs, and chick down feathers. Total mercury (THg) concentrations measured in whole blood are used to estimate MeHg concentrations found in tissues as 70-95% of THg is MeHg (Mortensen et al., 2014).

There is an abundance of literature regarding the effects of mercury in avian species. Frederick and Jayasena (2011) experimentally tested the effects of MeHg on courtship, pairing behavior, and breeding success in captive white ibises (*Eudocimus albus*); dosed groups had significantly more unproductive nests than non-dosed group (Frederick and Jayasena 2011). In common loons (*Gavia immer*), high adult blood mercury levels led to decreased time males and females spent incubating eggs and decreased time spent in high energy behaviors while brooding their young (Evers et al., 2008). During the 1970s, two

studies by Heinz (1974, 1979) found that MeHg in mallard ducks (*Anas platyrhynchos*) affected egg weight, “soundness” of eggs, hatching success, chick mortality within first week of life, and percent weight gain in mallard ducks as well as affecting reproductive success and behavior across generations.

Omnivorous birds such as whooping cranes could have a reaction behaviorally or physiologically to mercury, especially in small chicks or colts. As the EMP’s reproductive success continues to remain low, researchers continue to examine the cause for the low success. Contaminants such as mercury have the toxicity to affect both the adult birds and chicks in a multitude of ways. We broadly hypothesized that mercury, an anthropogenic environmental contaminant, could hinder the cranes’ ability to rear offspring to independence. This hypothesis stems from the fact that (1) mercury has been measured in Wisconsin breeding grounds and (2) when methylated, mercury may alter a wide range of behaviors including parental care, foraging, and reproductive success. To test this, we had to first document the levels of mercury in cranes from different populations. We predicted that the EMP would have higher levels of mercury as compared to the AWBP and International Crane Foundation population (ICFP). Thus, we measured total mercury in three whooping crane populations: AWBP, the EMP, and captive birds from the ICFP. This allowed us to compare birds of known successful reproduction (ICFP), birds of presumed successful reproduction (AWBP), and birds of known low reproduction (EMP).

Methods

Sample collection and analysis. Blood samples were pulled from existing blood banks held by ICFP and the Wisconsin Department of Natural Resources from the three populations: 21 from AWBP, 10 from ICFP, and 17 from EMP. The criteria set for the sample selection were (1) birds four years of age or older and (2) majority of samples collected during the fall season. The AWBP samples were collected during the winter season (December through February) as this was the only feasible time to access the birds. One or more ml from each sample was mixed with lithium heparin, an anticoagulant, and stored frozen (-20°C or -80°C) until analysis. All samples were collected by trained personnel and under valid federal permits.

The samples were sent to the Wisconsin State Laboratory of Hygiene (WSLH) for mercury analysis. The protocol used by the laboratory followed the Center for Disease Control (CDC) method of mercury analysis in humans (CDC Laboratory Procedures, 2016) altered for avian application used by WSLH. Avian blood sample analysis requires an additional step of heating because birds have nucleated erythrocytes, which can lead to varying degrees of agglutination. The samples were diluted with ammonium pyrolidine dithiocarbamate and tetramethyammonium hydroxide to optimize reliability of the measurement. To avoid agglutination and assure dissolution, the diluted samples were heated in a heating block to 75°C, and not exceeding 80°C, for 30-40 minutes. Once complete sample dissolution had occurred, samples were

analyzed following CDC protocol (CDC Laboratory Procedures) to provide a measure of Hg in parts per billion (ppb).

Statistical analysis. The aggregate Hg results for each population were compared with an ANOVA using R (R Core Team, 2017); post-hoc pairwise comparisons were performed with a standard Tukey HSD; 95% confidence intervals (CI) were calculated. To compare mean mercury levels as a function of sex within each population, a Welch two-sample t-test was performed. Due to variation in collection dates for each population and low sample size, a Kruskal-Wallis test was performed for each population to determine statistically significant differences in mercury as a function of month. As the EMP is the population of concern, a linear regression was performed to compare mercury concentrations as a function of age for those birds alone.

Descriptive analysis. The EMP whooping crane blood samples were collected during the months of August through November, during the years of 2008-2012, when the birds were at NNWR. There was one outlier for this fall collection period being bird 1-01; this sample was collected in May 2014 (Table 1). Individual ages ranged from four to 13 years old with an average age of 6.5 years. Seven male and 10 female samples were analyzed. The samples were stored in a blood bank at a WDNR facility in Madison, WI.

The ICFP whooping crane blood samples were collected throughout the year and stored in a blood bank at ICFP. The captive whooping cranes had an average age of 15.5 years (Table 1). The lifespan of a crane increases in

captivity; the individual ages ranged from four to 31 years old. Five male and five female blood samples were analyzed.

The AWBP blood samples were collected during the months of December through February, the period in which the birds are wintering at ANWR. The age of the birds is unknown as these birds are part of the self-sustaining wild migrating population, however the minimum age can be estimated at 1.5 years (Table 1). The samples collected from AWBP were from birds that had molted their juvenile sandy colored plumage; they are known as white-plumage adults. There were 11 male and 10 female blood samples analyzed.

Table 1

Bird blood sample information, listed for the collection period, age of the birds, sex ratio (male: female) and the mean mercury (parts per billion) values.

Population	Collection period	Age (mean, range)	Sex ratio M:F	Mercury ppb (mean)
ICFP	Throughout the year	15.5 years, 4-31	5:5	34.43
AWBP	Dec-Feb	White-plumage Adults	11:10	110.09
EMP	Aug-Nov	6.5 years, 4-13	7:10	347.7

Results

Population effect on mercury. Mercury levels differed significantly as a function of population ($F = 16.99$, $df = 2$, $p < 0.0001$, Figures 1 & 2). Pairwise comparisons revealed a difference between EMP and AWBP mercury levels, with EMP levels significantly higher than those observed in the AWBP (mean EMP = 347.7 ppb, mean AWBP = 110.09, 95% CI = [251.01 - 115.41], $p < 0.0001$, Figure 1). There was also a difference between ICFP and EMP mercury levels, with EMP levels significantly higher than ICFP levels (mean EMP = 347.7 ppb, mean ICFP = 34.43,95% CI = [-365.5 - -531.18], $p < 0.00001$, Figure 1). The pairwise comparison showed no significant difference between the AWBP and ICFP (95% CI (-114.54 - -274.2), $p = 0.20$, Figure 1).

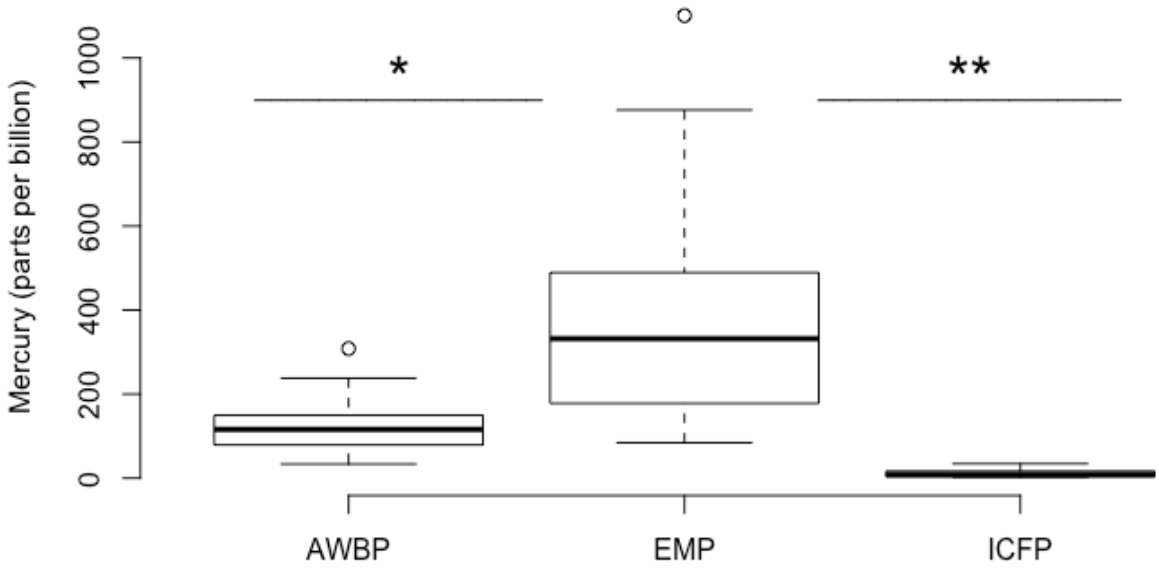

Figure 1. Boxplots comparing mean mercury values (parts per billion) between AWBP, EMP, and ICFP blood samples. Open circles represent outliers. Comparisons of AWBP (n=21) and EMP (n=17) (*p=0.001); EMP and ICFP (n=10) (**p< 0.0001); and AWBP and ICFP (p=0.20).

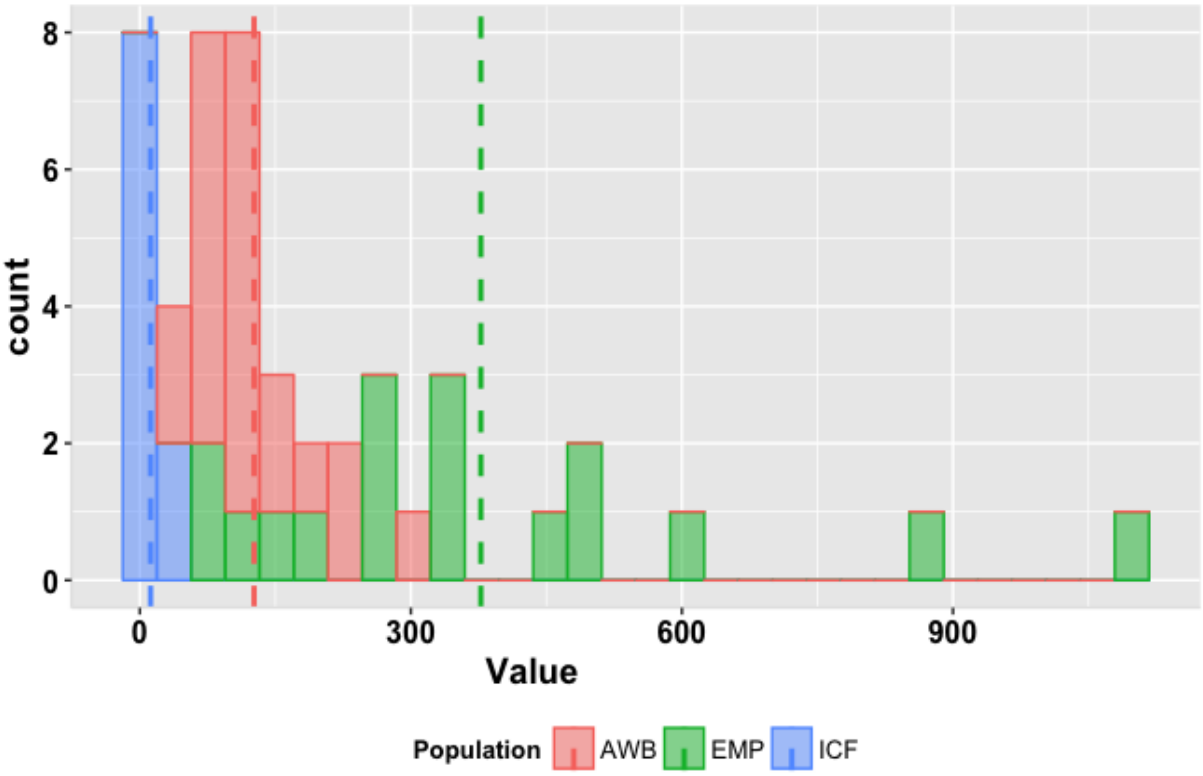

Figure 2. Histogram of individuals from each population and their measured mercury (parts per billion). The red bars represent the blood samples collected from the wild AWBP population. The green bars represent the blood samples from the reintroduced EMP. The blue bars represent the blood samples from the captive ICF population. The dashed lines represent the mean mercury (ppb) for each population, ICF =11.92, AWB = 110.09 EMP = 347.7. The y-axis is the count for the birds with a maximum number of birds at eight sharing a given interval of mercury values.

Comparisons for sex differences. To test whether or not there was a difference in mercury levels between sexes, we used a Welch two-sample t-test to compare means as a function of sex. For the AWBP there was a significant difference (mean= 110.09 ppb, 95% CI (21.12 - 128.19), p = 0.009, Figure 3), with males having higher mercury levels than females. Comparing male and

female mercury levels in the EMP, there was no significant difference (mean= 347.7 ppb, 95% CI (-360.6 - 233.49), p = 0.65, Figure 3). The ICFP also showed no significant difference in mercury levels as a function of sex (mean=34.43 ppb, 95% CI (-5.59 - 24.872), p = 0.17, Figure 3).

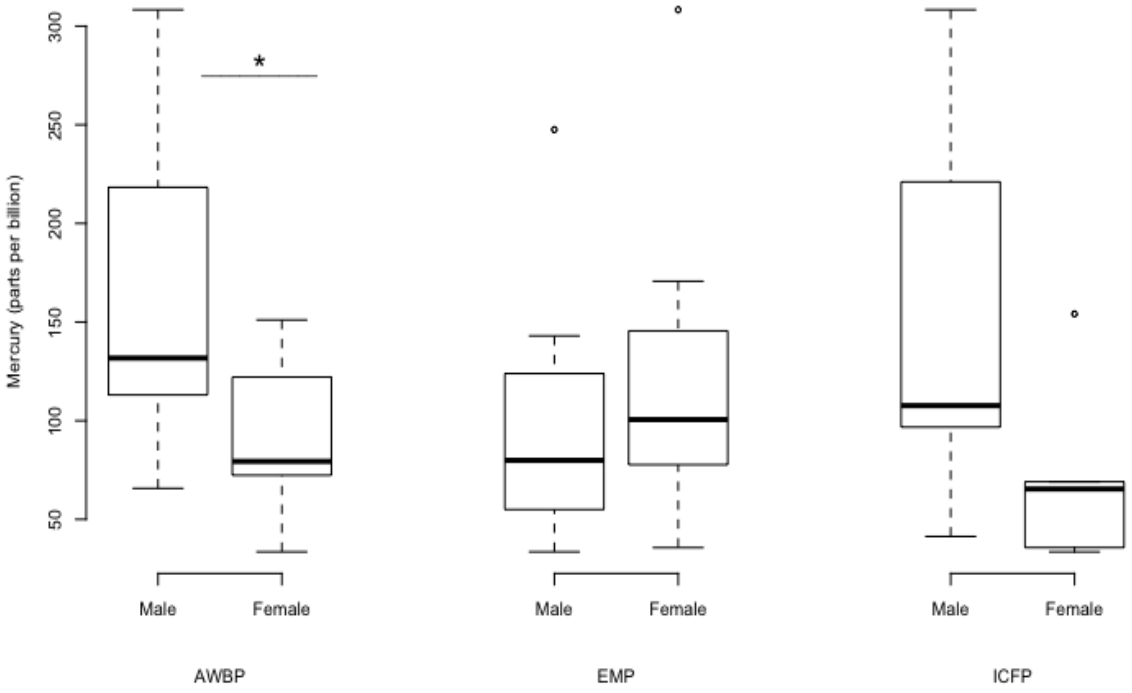

Figure 3. Boxplots comparing mean mercury values (parts per billion) as a function of sex for AWBP, EMP, and ICFP. Only males (n = 11) and females (n = 10) from AWBP significantly differed from one another (*p = 0.009). EMP males (n = 7) and females (n = 10) were not significantly different (p = 0.65). ICFP males (n = 5) and females (n = 5) were not significantly different (p = 0.17).

Monthly mercury averages by population. Due to variation in collection dates for each population, a non-parametric Kruskal-Wallis test was performed to determine if mercury concentrations varied monthly in the AWBP, ICFP, and EMP. The Kruskal-Wallis test showed no significant differences in mercury

concentrations by month for AWBP (p = 0.46), ICFP (p = 0.44), and the EMP (p = 0.45) (Figure 4).

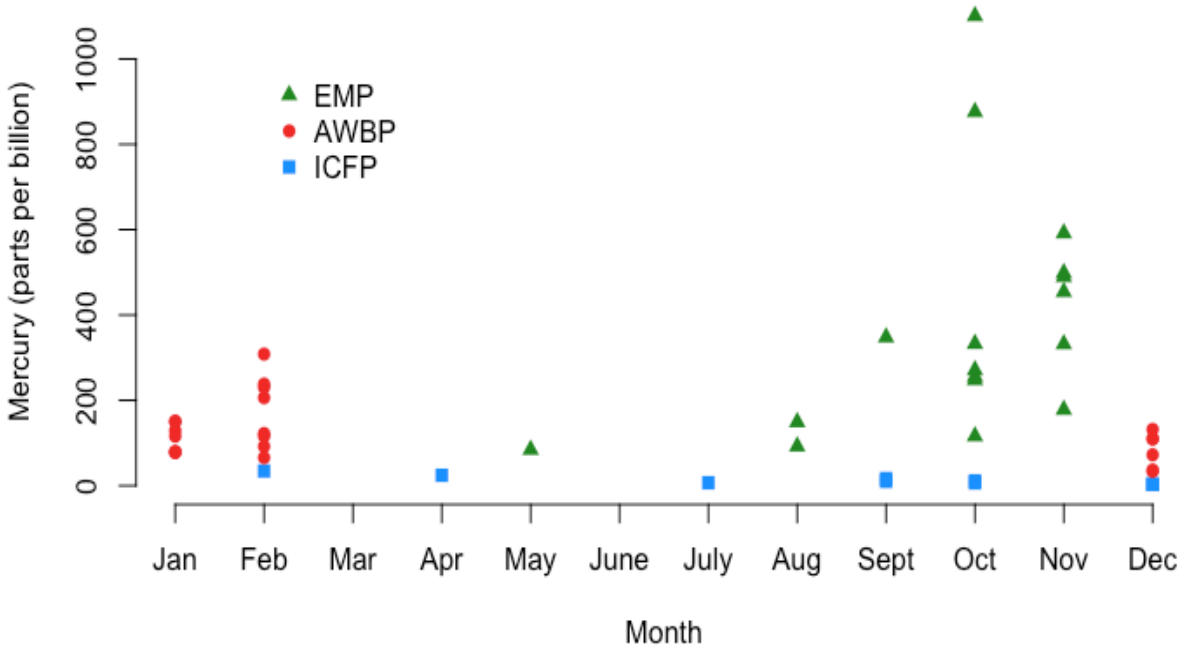

Figure 4. Individual mercury concentrations as a function of time.

Mercury concentrations as a function of age. To test mercury concentrations as a function of age in the EMP a linear regression was performed. There was no significant relationship between mercury ppb and the age of the EMP birds (p = 0.06, R^2 adjusted = 0.21, Figure 5).

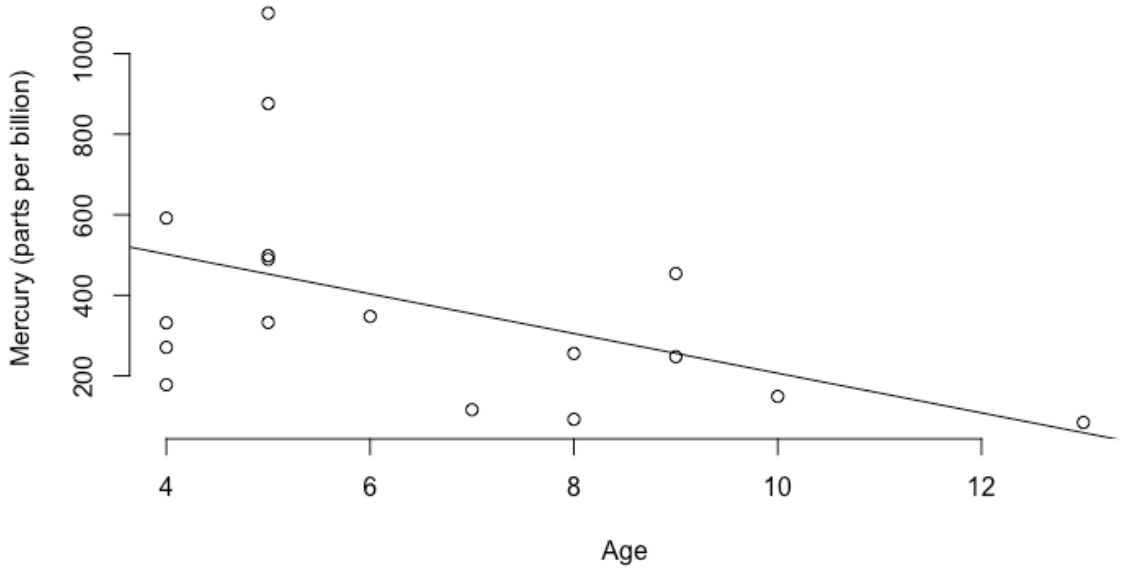

Figure 5. Individual mercury concentrations as a function of age for the EMP whooping cranes.

Discussion

The EMP is struggling as a population due to pairs' inability to rear chicks successfully to fledge (Urbanek et al., 2010). One hypothesis proposed to explain the high chick mortality is that parental care behaviors and offspring health are affected by exposure to mercury. Thus, we examined the levels of mercury in three populations of whooping cranes. We found a significant difference in mercury levels between the EMP, AWBP, and ICFP with EMP having significantly higher mercury levels than either ICFP or AWBP (Figures 1 & 2). The EMP and AWBP are both migratory populations, but the AWBP has presumed high reproductive success as the population is increasing (IUCN, 2016). The EMP has known low reproductive success, as the breeding pairs are unable to rear offspring to independence. ICFP birds have known high reproductive success in captivity (Urbanek et al., 2005). ICFP levels of mercury were barely detectable (Figures 1 & 2). Looking at sex differences within each population, only mercury levels in the AWBP birds differed significantly as a function of sex, with males having higher levels than females (Figure 3).

Limitations. These results are quite striking. There is, however, a discrepancy in the collection times of the samples. The majority of the EMP samples were collected during the months of August through November, and between 2008 and 2012. As the deposition of mercury can increase or decrease over time, one year may present higher or lower levels of mercury than a different year, even if the samples are from the same location (Ullrich et al.,

2001). The AWBP samples were collected December through February, in the years 2011 and 2014. This is the period when the birds are on the wintering grounds as opposed to the EMP samples, which were collected on the breeding summer grounds. The AWBP samples were collected during winter months because the birds were much more accessible at Aransas NWR as compared to Wood Buffalo National Park. When AWBP birds summer in Canada, researchers have difficulty getting to the birds, as Wood Buffalo National Park is highly secluded. Again, with the change in season, there may be a difference in the wet or dry disposition of MeHg (Ullrich et al., 2001). The ICFP samples were collected year-round during routine veterinary checks. Unsurprisingly, ICFP blood samples were very low in MeHg as this is a captive population on a diet provided to them by aviculturists.

Age of the birds could also be a confounding factor. All of the EMP and ICFP samples were from birds that were a minimum of four years of age. This ensured the birds were adults and capable of breeding, thus reproductively mature. We wanted breeding adults because the problem being examined is low reproductive success in the EMP. We could not define exact ages for AWBP and thus used plumage as an indicator. The AWBP birds were fledged white-plumage adults and thus presumed to be of breeding age, however these morphs can be as young as 1.5 years. At this age the birds are still considered juveniles as they will not be forming pairs or attempting to nest until approximately three to four years old (Urbanek, 2005). An important note is that

although we did use white-plumage birds in the AWBP, mercury remains in the birds' blood system for two to three months, regardless of the birds' age (Scheulhammer et al., 2007). The effects of mercury can affect birds of all ages: egg, chick, juvenile, adult (Walsh, 1990).

Another confounding factor is the potential for sampling bias. The EMP blood samples were not randomized for bird and then collected. Rather, the samples were collected as a means of convenience when the bird was captured, typically for transmitter replacement. This is similar to the birds at ICFP and AWBP. The birds at ICF represent a group of birds of varying age and the same number of males and females; the samples were collected during annual veterinary exams. The AWBP samples were collected during an examination of birds at the wintering grounds in Aransas; birds were captured based on availability. The collection of blood samples from the AWBP was more random than the EMP, as these birds were subadults but all the birds are of unknown age. We assume that each sample group represents the overall population from which it came.

Habitat differences. The goal of species conservation is to raise the number individuals in a population so that it will persist through hardships such as stochastic environmental events. However, endangered species' habitat is being threatened as anthropogenic demands for land increases.

To increase the survival probability of threatened and endangered species, reintroduction is a common practice to reestablish extirpated

populations or supplement dwindling ones (Sala et al., 2014). However, the habitat into which WHCR releases have occurred was determined by researchers based on the environments for which the AWBP showed preferences (Urbanek et al., 2005). The differences in the mercury levels between the ICFP, AWBP, and EMP can possibly be explained by the birds' habitat differences.

ICFP habitat. The captive birds at ICF are in a protected, controlled environment with the birds being provided an omnivorous diet throughout the year. ICF uses assisted reproduction to maximize fertility. The eggs are carefully monitored to have the highest hatchability rates of fertile eggs. The sheltering and managing of the birds in a captive situation likely leads to higher fledging rates than the AWBP.

AWBP habitat. The AWBP is a relictual wild population that was once widespread through North America (Mueller et al., 2013). Currently, the AWBP summers in remote shallow wetlands with poor drainage leading to potholes in which the cranes nest (Timoney, 1999). In contrast, the winter grounds are easily accessible to humans, and characterized by tidal flats, shallow bays, and estuarine marshes. When the birds are wintering at Aransas NWR, they are exposed to coastal industry with the Gulf Intracoastal Waterway in the vicinity. In a large mercury review on North American birds (Ackerman et al., 2016), ocean and estuary environments tended to have birds with higher blood-equivalent THg concentrations than in freshwater environments. The difference may be

explained by difference in MeHg bioavailability, which is affected by biogeochemical conditions, inorganic mercury availability, and mercury methylation rates (Ullrich et al., 2001, Ackerman et al., 2016). Species that belong to higher trophic level guilds tend to have higher levels of MeHg due being piscivorous and carnivorous (Ackerman et al., 2016). The AWBP birds have a very different diet at the Aransas winter grounds than at their summer grounds. Based on the ecoregion, habitat, and food choices of the AWBP birds, one could argue that AWBP birds would have more exposure to mercury compared to the EMP birds. However, the levels in the AWBP were significantly lower than the EMP birds.

EMP habitat. The EMP birds roost in freshwater, shallow open wetlands and forage in agriculture farmland (Channell & Lomolino, 2000). In the EMP, WHCR will venture from roost and nest sites on the refuge to neighboring agricultural land to forage seeds. This foraging behavior occurs in the AWBP during migration and wintering, but in the EMP, this behavior occurs throughout the year. The EMP also forages for seeds during migration, spanning from Illinois to Florida (WCEP, 2018). The EMP summers primarily in Wisconsin. Wisconsin is the 23rd largest state by size in the United States and of its 41.9 million acres; 14.3 million acres are designated as agricultural (Department of Agriculture, Trade and Consumer Protection, 2018). The EMP WHCRs are regularly exposed to agriculture and manufacturing industry.

One agricultural chemical in particular, the pesticide methyl iodide (also known as iodomethane), is a fumigant used as a pre-plant biocide used to control insects, plant parasitic nematodes, soil borne pathogens, and weed seeds (USEPA, 2007). Methyl iodide has been used as a methylating agent in the pharmaceutical industry, but in nature methyl iodide can convert organic mercury to the more toxic form of MeHg (National Center for Biotechnology Information, 2018). In laboratory studies of mammals, the brain is the primary organ affected by methyl iodide (Hallier et al., 1990). Although the negative effects of this pesticide are known, it is still widely used.

Along with agriculture, there is a renewal in manufacturing in the Midwestern United states. With an increase in industry comes an increase in amount of mercury being cycled, thus an increase in deposition (Schroeder & Munthe, 1998) and chances for contamination. The state of Wisconsin, the location of Necedah NWR, is top ten in the United States for manufacturing and industrial leaders (Crawford, 2013), which could have a direct impact on WHCR habitat.

The EMP's habitat could be the source of the mercury levels found in the blood samples as the birds could be exposed to mercury through their diet. If this is the case, we suggest that a prey sampling descriptive study be conducted to evaluate mercury levels in various species on the refuge. Along with the diet, the birds are in a wetland environment so water, and soil testing in the areas of most concern (roost sites, nest sites, etc.) could provide knowledge on the

exposure pathway of the whooping cranes to mercury at the Necedah NWR. In addition, aquatic mercury can enter a terrestrial food web, (Howie et al., 2018) leading to the potential for the EMP birds to get mercury from sources other than aquatic prey.

To test the hypothesis that differences between the populations is due to a difference in the exposure pathway, further environmental contaminant soil and water studies are necessary to provide useful knowledge on contaminant sources and levels.

Mercury studies. There is plentiful experimental and comparative research that examines the effects of MeHg physiology and behavior. However, little is known about how mercury affects cranes in particular and what the threshold values are for cranes. In addition, we do not understand the effects of low levels of mercury on physiology and behavior of cranes thus, we do not know if the ppm documented in the EMP is problematic.

Non-avian mercury studies. The bulk of data on the effects of MeHg toxicity on behavior and physiology has been done in non-avian systems; there is a consistency of results across taxa, however, which suggests exposure to mercury could lead to abnormal behavioral responses to predators or foraging opportunities. Although the data are limited in respect to the impacts of low levels of mercury in cranes, there is abundant research on this topic in humans. For example, researchers examined children who were prenatally exposed to MeHg, looking for the cognitive impacts of MeHg at blood levels that have been

previously deemed by toxicologists as “safe” (Grandjean et al., 1998). The study showed that on six different neuropsychological measures, a cohort of 112 seven-year-old children demonstrated mild abatements in motor function, language, and memory (Grandjean et al., 1998). The authors concluded that although the prenatal levels of MeHg were deemed “safe,” brain function declines in the children were detectable. In a different study, blood pressure, heart rate, and heart rate variability were measured in 1,000 seven-year-old human children (Sørensen et al., 1999). This study showed that prenatal exposure to MeHg might have affected the cardiovascular development and homeostasis in the subjects (Sørensen et al., 1999).

In mammals, MeHg toxicity causes central nervous system damage, including behavioral impairments (Wren et al., 1988). Non-human mammals have been used as model systems to study mercury toxicity in human infants. A study by Gunderson et al. (1986) tested the effects of low-level MeHg on infant crab-eating macaques. Through blood assays, the results showed the dosed macaques had significant recognition deficits and difficulties in visual attention to novel stimuli than the non-dosed control macaques (Gunderson et al., 1986). A second study evaluated the low dose effects of MeHg exposure on exploratory behavior, recognition memory, spatial learning, and acquisition of aversive memories in periadolescent and young adult rats (Albores-Garcia et al., 2016). These results suggest that low dose MeHg in blood can induce age-dependent negative effects as the periadolescent rats showed negative effects

on the test perimeters but the young adulthood littermates showed no effects (Albores-Garcia et al., 2016). The EMP whooping crane chicks may have low levels of MeHg, but even this might cause the EMP chicks to have difficulty in recognizing predators as the dangerous threats they represent.

Monitoring species of concern is difficult if the tests require tissues, which are obtained after the animal is deceased. Examining non-lethal biomarkers has been done on loggerhead sea turtles (*Caretta caretta*), an endangered species (Day et al., 2005). Researchers tested the ability of nonlethal matrices, blood, and keratinized scutes to predict internal tissue values. Blood samples were found to be an accurate predictor of THg in muscle and the spinal cord. In reptiles, scutes were found to be an accurate predictor of long-term exposure to mercury like what is seen in the liver. Given the results of this study, a potential study in birds could be conducted to examine whether or not bird feathers, also made from keratin, are a nonlethal biomarker for THg levels in the liver.

Avian mercury studies. There is a novel study on egret chicks (*Ardea alba*) exposed to low doses of MeHg. Egret chicks dosed with low levels of MeHg were found to be less likely to forage and they also had lower motivation to hunt for prey than non-dosed control chicks (Bouton et al., 1999). In white ibises (*Eudocimus albus*) with a measured blood THg level of 700 ppb, there was a 13% decrease in nest productivity and behaviorally the birds demonstrated altered courtship behavior (Frederick & Jayasena, 2011).

Using whole blood samples to test for mercury is not as common as using tissues or feathers. However, Ackerman et al., (2016) did a comprehensive literature review on avian mercury studies. They converted mercury levels from five different tissues (egg, muscle, liver, kidney, feathers) to blood-equivalent total mercury levels. This allows various matrices of mercury levels to be comparable as all mercury concentrations are calculated and converted to blood-mercury concentrations. In the double-crested cormorant (*Phalacrocorax auritus*), blood-equivalent THg was measured at 300 ppb, and the females demonstrated altered expression of XXX gene (Gibson et al., 2014). In the egg of a ring-necked pheasant, the blood-equivalent THg was calculated at 300 ppb and researchers found decreased egg hatchability (Spann et al., 1972 as cited in Ackerman et al., 2016). At 500 ppb in multiple species, Heinz et al., (2009) found an LC_{50} of egg hatchability. An LC_{50} is the concentration of mercury that will kill 50% of the eggs with a single exposure. In the Carolina wren (*Thryothorus ludovicianus*), whole blood was measured at 700 ppb. At these levels, researchers found a 10% reduction in the probability of nest success (Jackson et al., 2011). In their review, Shore et al. (2011) proposed 800 ppb as the indicative concentration of impaired reproduction across avian genera.

In the ICFP birds, the blood concentrations of mercury ranged from 3 - 35 ppb, with a mean of 11.92 ppb. These values are well below any negative effects of mercury documented in the literature. The AWBP birds had blood concentrations of mercury ranging from 33 - 306 ppb, with a mean of 110.09 ppb;

there was only one individual near or above 300 ppb. In the EMP birds, the blood concentrations of mercury ranged from 85-875 ppb, with a mean of 347.7 ppb. There were nine birds that were above 300 ppb, meaning these birds may have behavioral or physiological changes due to mercury toxicity. In the EMP, there were two outliers, a female measuring 876.06 ppb, and a male with 1100.77 ppb. Further environmental studies should be collected based on the preferred habitat location of these individuals.

Our study is novel in that whole blood samples were measured for a baseline in multiple populations of endangered whooping cranes. The results show low levels of mercury in all WHCR populations; however, there is no agreement on what a safe threshold is for humans let alone other mammals or birds. The effects of MeHg on the chicks and adults may result in explicit deficiencies in the EMP compared to the lower levels of Hg in the self-sustaining AWBP WHCR. The birds' environment is crucial to their continued survival, which is why some of their habitat has been federally protected. Although the inability of EMP birds to rear chicks to fledge is likely a multifaceted problem, contaminants could be one part in the change in behavior or physiology leading to low reproductive success. More research is needed to examine the sub lethal effects contaminants such as mercury have on *G. americana.*

Chapter IV:
CONCLUSIONS

Determining whether contaminants could be a cause of the low reproductive success observed in the EMP first requires knowledge of contaminant levels in their habitat as compared to the habitat of other WHCR populations.

One hypothesis proposed to explain the EMPs low reproductive success is that the birds have been exposed to relatively high levels of mercury. To begin addressing this, this researcher tested mercury levels in three WHCR populations: the AWBP, EMP, and ICFP. This allowed comparing mercury levels across birds of known successful reproduction (ICFP), birds of presumed successful reproduction (AWB), and birds of known low reproduction (EMP). Blood samples were obtained from the Wisconsin Department of Natural Resources (EMP) and the International Crane Foundation (AWBP, ICFP). Levels of methylmercury were then analyzed at the Wisconsin State Laboratory of Hygiene. The analysis showed that, although the mercury levels in the EMP were, on average, low compared to other avian species, they were significantly higher than levels observed in AWBP and ICFP blood samples. These data suggest that although there are not toxic thresholds in the EMP WHCRs, mercury may have underlying and sublethal effects on reproductive success.

Further research on low levels of mercury and its effects on reproduction are needed, specifically in birds, as the literature is limited in this area. Low

levels of mercury have been shown to have negative effects on a wide range of behaviors (Gunderson et al., 1986, Balazs and Pooley, 1991, Wren et al., 1988, Walsh, 1990, Sørensen et al., 1999).

Management Implications

This was a novel study measuring mercury in three separate populations of whooping cranes. More research needs to continue in the field of contaminants, as eco-toxicants continue to increase with the increase in detrimental anthropogenic activities. The continuation of research on this charismatic and endangered species can help to improve management and conservation efforts to prevent their extinction.

APPENDIX A

Bird Blood Sample Information

ID	Collection Date	Population	Age	Sex	Value (ppb)
2011-06	12/8/11	AWBP	Adult	M	33.56
2011-03	12/6/11	AWBP	Adult	F	72.36
2011-07	12/7/11	AWBP	Adult	M	109.7
2011-09	12/8/11	AWBP	Adult	F	110.09
2011-05	12/7/11	AWBP	Adult	M	131.75
2011-02	12/6/11	AWBP	Adult	F	36.69
2014-51	2/2/14	AWBP	Adult	M	238
2014-82	1/17/14	AWBP	Adult	F	116.07
2014-45	1/11/14	AWBP	Adult	F	230.41
2014-46	2/3/14	AWBP	Adult	M	151.1
2014-48	1/10/14	AWBP	Adult	F	116.03
2014-44	2/1/14	AWBP	Adult	M	79.7
2014-54	2/3/14	AWBP	Adult	M	79.04
2014-50	2/2/14	AWBP	Adult	M	129.15
2012-39	1/17/14	AWBP	Adult	M	308.29
2014-49	1/17/14	AWBP	Adult	F	206.2
2014-52	2/4/14	AWBP	Adult	F	92.01
2014-53	1/12/14	AWBP	Adult	M	149.57
2011-12	2/3/14	AWBP	Adult	M	65.72
2014-47	1/15/14	AWBP	Adult	F	122.13
2014-56	2/1/14	AWBP	Adult	F	77.63
13-117	2/20/14	ICFP	18	M	9.99
13-144	9/28/17	ICFP	18	F	34.43
13-115	9/17/12	ICFP	16	M	16.62

ID	Collection Date	Population	Age	Sex	Value (ppb)
13-27	4/12/12	ICFP	23	M	6.37
13-281	12/3/10	ICFP	5	F	6.79
13-205	7/17/14	ICFP	9	F	24.35
13-19	10/27/16	ICFP	31	F	3.59
13-40	10/4/10	ICFP	26	M	2.69
13-282	12/3/10	ICFP	4	F	2.94
13-280	12/3/10	ICFP	5	M	11.25
16-02	9/30/08	EMP	6	M	84.31
09-03	11/4/08	EMP	5	F	92.16
17-02	10/19/09	EMP	7	F	116.02
06-05	11/3/09	EMP	4	M	149.08
19-05	11/11/09	EMP	4	F	178.07
12-04	11/13/09	EMP	5	M	247.62
17-07	10/15/11	EMP	4	F	255.76
11-02	8/19/12	EMP	10	M	270.84
16-07	10/12/12	EMP	5	F	331.87
13-03	10/17/12	EMP	9	F	332.66
03-07	10/23/12	EMP	5	M	347.7
02-04	10/24/12	EMP	8	M	454.11
26-07	10/24/12	EMP	5	F	489.31
24-08	11/1/12	EMP	4	F	498.52
09-03	11/5/12	EMP	9	F	591.77
3-03	8/16/11	EMP	8	F	876.06
1-01	5/28/14	EMP	13	M	1100.77

REFERENCES

Ackerman, J. T., Eagles-Smith, C. A., Herzog, M. P., Hartman, C. A., Peterson, S. H., Evers, D. C., Jackson, A.K., Elliott, J.E., Vander Pol, S.S., Bryan, C. E. (2016). Avian mercury exposure and toxicological risk across western North America: A synthesis. *The Science of the Total Environment*, *568*, 749–769.

Albores-Garcia, D., Acosta-Saavedra, L., Hernandez, A. J., Loera, M. J., Calderón-aranda, E. S. (2016). Early developmental low-dose methylmercury exposure alters learning and memory in periadolescent but not young adult rats. *Biomed Research International*, *2016*, 12 pp.

Allen, R. P. (1952). *The Whooping Crane*. New York, NY: National Audubon Society.

Balazs, G.H., Pooley, S.G. (1991). Honolulu: National Oceanographic and Atmospheric Administration, National Marine Fisheries Service. Research Plan for Marine Turtle Fibropapilloma. Report No. NOAA-TM-NMFS-SWFSC-156.

Bolate, Y. (2017). Inventory of U.S. sources of mercury emissions to the atmosphere. *Columbia University;* 1–29.

Bouton, S. N., Frederick, P. C., Spalding, M. G., McGill, H. (1999). Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environmental Toxicology and Chemistry*, *18*(9), 1934–1939.

Brasso, R., & Cristol, D. A. (2008). Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). *Ecotoxicology*, *17*(2), 133–141.

Burgess, N. M., & Meyer, M. W. (2008). Methylmercury exposure associated with reduced productivity in common loons. *Ecotoxicology*, 17(2), 83–91.

Canadian Wildlife Service and U.S. Fish and Wildlife Service. (2006). International Recovery Plan for the Whooping Crane (*Grus americana*) (Revised). Environment Canada, Ottawa, and U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 162 pp.

Canadian Wildlife Service and U.S. Fish and Wildlife Service. (2007). International recovery plan for the whooping crane. Ottawa: Recovery of Nationally Endangered Wildlife (RENEW), and U.S. Fish and Wildlife Service, Albuquerque, New Mexico. 162 pp.

Cannon, J. R. (1999). Wisconsin Whooping Crane Breeding Site Assessment - Final Report. Submitted to the Canadian-United States Whooping Crane Recovery Team, September 22, 1999.

Celo, V., Lean, D. R. S., Scott, S. L. (2006). Abiotic methylation of mercury in the aquatic environment. *Science of the Total Environment*, *368*(1), 126–137.

Centers for Disease Control and Prevention (CDC) Laboratory Protocol. Hyattsville, MD: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, [2016] [https://wwwn.cdc.gov/nchs/data/nhanes/2015-2016/labmethods/PBCD_I_met.pdf].

Channell, R., & Lomolino, M. V. (2000). Dynamic biogeography and conservation of endangered species. *Nature*, *403*(6765), 84–86.

Cheng, Z., Liang, P., Shao, D. D., Wu, S. C., Nie, X. P., Chen, K. C., Li, K.B., Wong, M. H. (2011). Mercury biomagnification in the aquaculture pond ecosystem in the Pearl River delta. *Archives of Environmental Contamination and Toxicology*, *61*(3), 491–499.

Condon, A.M., & Cristol, D.A. (2009). Feather growth influences blood mercury level of young songbirds. *Environmental Toxicology and Chemistry, 28*(2), 395–401.

Crawford, M. (Winter, 2013). *The states leading the U.S. manufacturing resurgence.* Retrieved from http://www.areadevelopment.com/RegionalReports/Q1-2013/states-leading-US-manufacturing-resurgence-2665542.shtml

Daso, A. P., Okonkwo, J. O., Jansen, R., Forbes, P. B. C., Kotzé, A., Rohwer, E. R. (2015). Mercury concentrations in eggshells of the Southern Ground-Hornbill (*Bucorvus leadbeateri*) and Wattled Crane (*Bugeranus carunculatus*) in South Africa. *Chemosphere*, *118*(1), 284–292.

Day, R., Christopher, J.S., Becker, P., Whitaker, D. (2005). Monitoring mercury in the loggerhead sea turtle, *Caretta caretta*. *Environmental science & technology, 39*(2), 437-446.

Department of Agriculture, Trade and Consumer Protection (2018, March 1). Wisconsin agricultural statistics. Retrieved from: https://datcp.wi.gov/Pages/Publications/WIAgStatistics.aspx

Driscoll, C. T., Han, Y.-J., Chen, C. Y., Evers, D. C., Lambert, K. F., Holsen, T. M., Kamman, N.C., Munson, R. K. (2007). Mercury contamination in forest and freshwater ecosystems in the Northeastern United States. *BioScience*, *57*(1), 17–28.

Erickson, R.C. & Derrickson, S.R. (1981). The Whooping Crane. In J.C. Lewis, H. Masatomi (Eds.), *Crane Research Around the World: Proceedings of the International Crane Symposium Sapporo, in Japan 1980 and Papers from the World Working Group on Cranes, International Council for Bird Preservation* (Third Edition, pp. 102-130). Baraboo, WI: International Crane Foundation.

Evers, D. C., Savoy, L. J., DeSorbo, C. R., Yates, D. E., Hanson, W., Taylor, K. M., Siegel, L.S., Cooley, J.H., Bank, M.S., Major, A., Munney, K., Mower, B.F., Vogel, H.S., Schoch, N., Pokras, M., Goodale, M.W., Fair, J. (2008). Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology*, *17*(2), 69–81.

Evers, D. C., Taylor, K. M., Major, A., Taylor, R. J., Poppenga, R. H., Scheuhammer, A. M. (2003). Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology*, *12*(1–4), 69–81.

Fleming, E. J., Mack, E. E., Green, P. G., Nelson, D.C. (2006). Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied and Environmental Microbiology*, *72*(1), 457–464.

Folk, M.J., Rodgers, J.A., Dellinger, T.A., Nesbitt, S.A., Parker, J.M., Spalding, M.G., Baynes, S.B., Chappell, M.K., Schwikert, S.T. (2008). Status of non-migratory whooping cranes in Florida. *Proceedings of the 11th North American Crane Working Group*, 118-123.

Frederick, P. & Jayasena, N. (2011). Altered pairing behaviour and reproductive success in white ibises exposed to environmentally relevant concentrations of methylmercury. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1713), 1851–7.

Gibson, L.A., Lavoie, R.A., Bissegger, S., Campbell, L.M., Langlois, V.S. (2014). A positive correlation between mercury and oxidative stress-related gene expression (GPX3 and GSTM3) is measured in female double-crested cormorant blood. *Ecotoxicology* 23, 1004– 14.

Glenn, T. C., Stephan, W., Braun, M. J. (1999). Effects of a Population Bottleneck on Whooping Crane Mitochondrial DNA Variation. *Conservation Biology* 13(5):1097-1107.

Grajewska, A., Falkowska, L., Szumiło-Pilarska, E., Hajdrych, J., Szubska, M., Frączek, T., Meissner, W., Bzoma, S., Beldowska, M., Przystalski, A., Brauze, T. (2015). Mercury in the eggs of aquatic birds from the Gulf of Gdansk and Wloclawek Dam (Poland). *Environmental Science and Pollution Research*, *22*(13), 9889–9898.

Grandjean, P., Weihe, P., White, R. F., Debes, F. (1998). Cognitive performance of children prenatally exposed to “safe” levels of methylmercury. *Environmental Research*, *77*(2), 165–172.

Gomez, G. M. (1991). Whooping cranes in southwest Louisiana: history and human attitudes. *North American Crane Workshop Proceedings*, 19–23.

Gu, B., Bian, Y., Miller, C. L., Dong, W., Jiang, X., Liang, L. (2011). Mercury reduction and complexation by natural organic matter in anoxic environments. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(4), 1479-83.

Gunderson, V., Grant, K., Burbacher, T., Fagan, J., Mottet, N. (1986). The effect of low-level prenatal methylmercury exposure on visual recognition memory in infant crab-eating macaques. *Child Development, 57*(4), 1076-1083.

Hallier, E., Deutschmann, S., Reichel, C., Bolt, H. M., Peter, H. (1990). A comparative investigation of the metabolism of methyl bromide and methyl iodide in human erythrocytes. *Environmental Health* 221–225.

Harrell, W. & M. Bidwell. (2016). Report on Whooping Crane Recovery Activities (2015 breeding season-2016 spring migration). United States Fish and Wildlife Service.

Hartup, B. K. In press. Rearing and release methods for reintroduction of captive-reared whooping cranes. In J. B. French, S. J. Converse, J. A. Austin, (Eds). The Biology and Conservation of the whooping crane (*Grus americana*). *Elsevier Inc.*

Heinz, G.H. (1974). Effects of low dietary levels of methylmercury on mallard reproduction. *Bulletin of Environmental Contamination and Toxicology*, *11*(4), 386–392.

Heinz, G.H. (1979). Methylmercury: reproductive and behavioral effects on three generations of mallard ducks. *The Journal of Wildlife Management*, *43*(2), 394.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R. (2009). Rapid increases in mercury concentrations in the eggs of mallards fed methylmercury. *Environmental Toxicology and Chemistry 28*, 1979–1981.

Howie, M.G., Jackson, A.K., Cristol, D.A. (2018). Spatial extent of mercury contamination in birds and their prey on the floodplain of a contaminated river. *Science of The Total Environment*, *630*, 1446–1452.

Hunter, J.G., Burger, J., Cooper, K.R. (2003). Use of an integrated mercury food web model for ecological risk assessment. *Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering*, *38*(7), 1201–1214.

International Crane Foundation (2018, November 13). Whooping crane. Retrieved from: https://www.savingcranes.org/species-field-guide/whooping-crane/

Jackson, A.K., Evers, D.C., Etterson, M.A., Condon, A.M., Folsom, S.B., Detweiler, J., Schmerfeld, J., Cristol, D.A. (2011). Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina wren (*Thryothorus ludovicianus*). *Auk* 128, 759–769.

King, R.S., McKann, P.C., Gray, B.R., Putnam, M.S. (2015) Host–parasite behavioral interactions in a recently introduced, whooping crane population. *Fish and Wildlife Management* 6(1): 220-226.

Lin, C. J., & Pehkonen, S.O. (1999). The chemistry of atmospheric mercury: A review. *Atmospheric Environment*, *33*(13), 2067–2079.

Luo, J., Ye, Y., Wang, Y. (2014). Dietary exposure of the red-crowned crane (*Grus japonensis*) to total and methyl mercury in Zhalong Wetland, northeastern China. *Biological Trace element Research, 159*(1-3), 210-218.

Mortensen, M. E., Caudill, S. P., Caldwell, K. L., Ward, C. D., Jones, R. L. (2014). Total and methyl mercury in whole blood measured for the first time in the U.S. population: NHANES 2011-2012. *Environmental research*, *134*, 257-64.

Mueller, T., O'Hara, R.B., Converse, S.J., Urbanek, R.P., Fagan, W.F. (2013). Social learning of migratory performance. *Science* (*339*)1335–1338.

National Center for Biotechnology Information. PubChem Compound Database; CID= 6328, https://pubchem.ncbi.nml.nih.gov/compound/6328 (accessed Mar. 29, 2018).

Ofukany, A.F.A., Hobson, K.A., Wassenaar, L.I. (2012). Connecting breeding and wintering habitats of migratory piscivorous birds: Implications for tracking contaminants (Hg) using multiple stable isotopes. *Environmental Science and Technology*, *46*(6), 3263–3272.

Operation Migration. (2018). *Our work.* Retrieved from http://operationmigration.org/the-work-of-operation-migration.asp

Pacyna, E.G., Pacyna, J.M., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F., Maxson, P. (2010). Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. *Atmospheric Environment*, *44*(20), 2487–2499.

Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D. (2002). Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proceedings of the National Academy of Sciences*, *99*(7), 4419–4423.

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

Rattner, B.A., Scheuhammer, A.M., Elliott, J.E. (2011). History of wildlife toxicology and the interpretation of contaminant concentrations in tissue. In W. N. Beyer & J. P. Meador (Eds.), *Environmental Contaminants in Biota: Interpreting Tissue Concentrations* (Second Ed, pp. 9–44). Boca Raton, FL: Taylor & Francis Group.

Rice, K.M., Walker, E.M., Wu, M., Gillette, C., Blough, E.R. (2014). Environmental mercury and its toxic effects. *Journal of Preventive Medicine and Public Health*, *47*(2), 74–83.

Rouse, J.G., & Van Dyke, M.E. (2010). A review of keratin-based biomaterials for biomedical applications. *Materials*, *3*(2), 999–1014.

Sadowski, C.L., Olsen, G.H., McPhee, M.E. (2018). Effects of rearing environment on behavior of captive-reared whooping cranes. *Proceedings of the North American Crane Workshop*,14:56-66.

Sala, O.E., Parton, W.L., Joyce A., Lauenroth W.K. (2014). Demography of a reintroduced population: moving toward management models for an endangered species, the whooping crane. *Ecological Applications 69*(1), 40–45.

Scheulhammer, A.M., Meyer M.W., Sandhein-rich M.B., Murray, M.W. (2007). Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–18.

Schroeder, W.H & Munthe, J. (1998). Atmospheric mercury: an overview. *Atmosphere and Environment, 32*:809–22

Selin, N. E. (2009). Global biogeochemical cycling of mercury: a review. *Annual Review of Environment and Resources*, *34*(1), 43–63.

Shore, R.F., Pereira, G.M., Walker, L.A., Thompson, D.R., (2011). Mercury in nonmarine birds and mammals. In W. N. Beyer & J. P. Meador (Eds.), *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*, 2:609-626.

Sørensen, N., Murata, K., Budtz-Jørgensen, E., Weihe, P., Grandjean, P. (1999). Prenatal methylmercury exposure as a cardiovascular risk factor at seven years of age. *Epidemiology;* 10:370-375.

Spann, J.W., Heath, R.G., Kreitzer, J.F., Locke, L.N. (1972). Ethyl mercury p-toluene sulfonanilide: lethal and reproductive effects on pheasants. *Science* 175, 328–331.

Teraoka, H., Okamoto, E., Kudo, M., Nakayama, S.M., Ikenaka, Y., Ishizuka, M., Endo, T., Kitazawa T., Hiraga, T. (2015). Accumulation properties of inorganic mercury and organic mercury in the red-crowned crane *Grus japonensis* in east Hokkaido, Japan. *Ecotoxicology and Environmental Safety*, *122*, 557–564.

The IUCN Red List of Threatened Species. Version 2016-2. < www.iucnredlist.org >. Downloaded March 22, 2018.

Timoney, K. (1999). The habitat of nesting whooping cranes. *Biological Conservation* 89:189-197.

Ullrich, S.M., Tanton, T.W., & Abdrashitova, S.A. (2001). Mercury in the aquatic environment: a review of factors affecting methylation. *Critical Reviews in Environmental Science and Technology*, *31*(3), 241–293.

Urbanek, R.P., Fondow, L.A., Satyshur, C.D., Lacy, A.E., Zimorski, S.E., & Wellington, M. (2005). First cohort of migratory whooping cranes reintroduced to eastern North America: the first year after release. In Chavez-Ramirez, F, ed. 2005. *Proceedings of the Ninth North American Crane Workshop,* 213-224.

Urbanek, R.P., Fondow, L.E.A., Zimorski, S.E., Wellington, M.A., and Nipper, M.A. (2010). Winter release and management of reintroduced migratory whooping cranes *Grus americana*. *Bird Conservation International* 20(1): 43-54.

U.S. Environmental Protection Agency. (1997). Mercury Study Report to Congress, vols. 1–8. Washington (DC): Office of Air Quality Planning and Standards and Office of Research and Development. Report no. EPA-452/R-97-005.

U.S. Environmental Protection Agency (USEPA) Pesticide Fact Sheet: Iodomethane. Office of Prevention, Pesticides and Toxic Substances [2007] [https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-000011_01-Jan-07.pdf]

U.S. Fish and Wildlife Services. (1997). U.S. Fish and wildlife service designates rocky mountain population of whooping cranes as “experimental”. Fish and Wildlife Service's Southwest Regional Office in Albuquerque, New Mexico.

U.S. Fish and Wildlife Service. (1994). Whooping Crane Recovery Plan. Albuquerque, New Mexico. 92 pp.

Walsh, P.M. (1990). The use of seabirds as monitors of heavy metals in the marine environment. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press, New York

Ward, D. M., Nislow, K. H., Folt, C. L. (2010). Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Annals of the New York Academy of Sciences*, *1195*, 62–83.

Whooping Crane Eastern Partnership (2018, February 1). Whooping crane update. Retrieved from: https://www.bringbackthecranes.org/technicaldatabase/projectupdates/2018/01Feb2018.html

Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A. (1998). Effects of mercury on wildlife: a comprehensive review. *Environmental Toxicology and Chemistry,* 17:146–160.

Wren, C.D., Fischer, K.L., Stokes, P.M. (1988). Levels of lead, cadmium and other elements in mink and otter from Ontario, Canada. *Environmental Pollution* 52:193-202.

Zillioux, E.J., Porcella, D.B., Benoit, J.M. (1993). Mercury cycling and effects in freshwater wetland ecosystems. *Environmental Toxicology and Chemistry*, *12*(12), 2245–2264.