Lead in species of greatest conservation need: free-flying bald eagles as indicators

by

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TABLE OF CONTENTS

Page

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CHAPTER 1. GENERAL INTRODUCTION

BACKGROUND

In Iowa, Bald Eagles (*Haliaeetus leucocephalus*) have increased in recent times from one nest in 1977 to more than 241 nests in 2013 (Dyar 2010, Shepherd 2013). However, many aspects of their biology, behavior, and the factors that influence their survival and reproduction have yet to be studied. In recent years, there has been a growing concern about whether lead ammunition is linked to lead exposure in Bald Eagles (New York Times 2010, Sheridan 2012). Raptor rehabilitators throughout the U.S. continue to report cases of lead exposure in Bald Eagles and other raptors and have also noticed temporal correlations between the incidence of lead exposure and the timing of upland and big game hunting seasons (Kramer and Redig 1997, Harris and Sleeman 2007, Neumann 2009, Redig et al. 2009, Strom et al. 2009, Stauber et al. 2010). In Iowa, between January 2004 and December 2013, nearly 60% of all Bald Eagles admitted to rehabilitation facilities had detectable levels of lead in their blood (Neumann, personal communication). Yet, little is known about the magnitude of lead exposure in free-flying Bald Eagles and whether those levels vary temporally or spatially. In addition, it is unknown whether lead levels in Bald Eagles admitted to rehabilitation centers are representative of lead levels in free-flying Bald Eagles.

In addition to concerns about lead exposure in Iowa Bald Eagles, as this species has increased in Iowa there is interest in understanding factors associated with their reproductive success. Reproductive success in Bald Eagles can depend upon a variety of factors including food abundance, weather conditions, location, habitat quality, age of adults, number of years holding a nesting territory, size of adult males, environmental contaminants (such as pesticides, heavy metals, and PCB's), and human disturbance (Newman et al 1977, McEwan

and Hirth 1979, Swenson et al. 1986, Hansen 1987, Anthony and Isaacs 1989, Grubb and King 1991, Bowerman et al. 1995, Steidl and Anthony 1996, Steidl et al. 1997, Elliot et al. 1998, Millsap et al. 2004, Jenkins and Jackman 2006). In Iowa, yearly surveys conducted by volunteer monitors help quantify the number of active nests and estimate the number of young fledged each year (Shepherd 2013). Still, little is known about the nesting, disturbance, and environmental parameters that influence Bald Eagle nest survival in Iowa. In raptors, any human activity that disrupts their normal behavior is considered a disturbance (Richardson and Miller 1997). The Bald Eagle is especially problematic because special permits are required to do research in the proximity of eagle nests, yet little is known about their reproductive success in the presence of human disturbance (Mathisen 1968, Grier 1969, Wood and Collopy 1993, Steidl and Anthony 2000).

GOALS AND OBJECTIVES

The primary goal of my study was to investigate dietary lead exposure in free-flying wintering and nesting Bald Eagles in Iowa. A secondary goal was to identify factors associated with nest survival. These goals were achieved by addressing these three objectives:

1. Characterize lead levels in the feces of nesting and wintering Bald Eagles in Iowa and identify temporal and spatial factors related to elevated lead exposure as a function of diet.
2. Compare lead exposure in free-flying eagles with eagles admitted to rehabilitation centers.
3. Characterize and identify factors that influence nest survival of Bald Eagle in Iowa.

THESIS ORGANIZATION

This thesis is organized into five chapters, of which the middle three are written in manuscript format. Above, I provided a general introduction to the material covered in the thesis (Chapter 1). Next, I present the results of a study investigating the extent of lead exposure in free-flying Bald Eagles in Iowa (Chapter 2). This is followed by a paper comparing lead exposure between free-flying Bald Eagles and Bald Eagles admitted to rehabilitation centers (Chapter 3). The final chapter is a paper describing the factors that influence nest survival in Bald Eagles in Iowa (Chapter 4). I end with a short summary of general conclusions and recommendations from the results of earlier chapters (Chapter 5). Manuscript authors each contributed to the design, data analyses, or writing of one or more papers.

LITERATURE CITED

Anthony, R.G., and F.B. Isaacs. 1989. Characteristics of bald eagle nest sites in Oregon. Journal of Wildlife Management 53:148-159.

Bowerman, W.W., J.P. Giesy, D.A. Best, and V.J. Kramer. 1995. A review of factors affecting productivity of bald eagles in the Great Lakes region: Implications for Recovery. Environmental Health Perspectives 103:51-59.

Dyar, J. 2010. Bald Eagles in Iowa. Iowa Department of Natural Resources

Elliot, J.E., I.E. Moul, and K.M. Cheng. 1998. Variable Reproductive Success of Bald Eagles on the British Columbia Coast. Journal of Wildlife Management 62:518-529.

Grier, J.W. 1969. Bald eagle behavior and productivity responses to climbing nests. Journal of Wildlife Management 33:961-966.

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.

Hansen, A. J. 1987. Regulation of bald eagle reproduction rates in Southeast Alaska. Ecology 68:1387-1392.

Harris, M. C. and J. M. Sleeman. 2007. Morbidity and mortality of Bald Eagles (*Haliaeetus leucocephalus*) and Peregrine Falcons (*Falco peregrinus*) admitted to the wildlife center of Virginia, 1993–2003. Journal of Zoo and Wildlife Medicine 38:62-66.

Jenkins, J.M., and R.E. Jackman. 2006. Lifetime Reproductive Success of Bald Eagles in Northern California. The Condor 108:730-735.

Kramer, J. L., and P. T. Redig. 1997. Sixteen years of lead poisoning in eagles, 1980-95: an epizootiologic view. Journal of Raptor Research 31:327-332.

Mathisen, J. E. 1968. Effects of human disturbance on nesting of bald eagles. Journal of Wildlife Management 32:1-6.

McEwan, L.C., and D.H. Hirth. 1979. Southern bald eagle productivity and nest site selection. Journal of Wildlife Management 43:585-594.

Millsap, B., T. Breen, E. McConnell, T. Steffer, L. Phillips, N. Douglas, and S. Taylor. 2004. Comparative fecundity and survival of bald eagles fledged from suburban and rural natal areas in Florida. Journal of Wildlife Management 68:1018-1031.

New York Times. 2010. The bullet that keeps on killing, editorial, Sept. 13, 2010.

Newman, J.R., W.H. Brennan, and L.M. Smith. 1977. Twelve-year changes in nesting patterns of bald eagles (*Haliaeetus leucocephalus*) on San Juan Island, Washington. The Murrelet 58:37-39.

Neumann, K. 2009. Bald Eagle lead poisoning in winter. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0119.

Neumann, K. 2014. Personal communication.

Redig, P. T., D. R. Smith, and L. Cruz-Martinez. 2009. Potential sources of lead exposure for Bald Eagles: A retrospective study. Extended abstract in R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0208.

Richardson, C. T., and C. K. Miller. 1997. Recommendations for protecting raptors from human disturbance: a review. Wildlife Society Bulletin 25:634-638.

Shepherd, S. 2013. Bald eagle (*Haliaeetus leucocephalus*) nesting in Iowa 2013. Iowa Department of Natural Resources. <http://www.iowadnr.gov/Portals/idnr/uploads/wildlife/research/BENestSummary_2012.pdf>. Accessed 28 January 2014.

Sheridan, K. 2012. Bald Eagle in crosshairs of US fight over lead bullets, editorial, Associated Free Press. April 6, 2012. <http://www.google.com/hostednews/afp/article/ALeqM5iLMsEXWCoy78F_AZc0aDOfGpruHw?docId=CNG.1862d6191b237577b6f5f5b8cdfd5d8e.f1>. Accessed 30 May 2012.

Stauber, E., N. Finch, P. a. Talcott, and J. M. Gay. 2010. Lead poisoning of bald (*Haliaeetus leucocephalus*) and golden (*Aquila chrysaetos*) eagles in the U.S. Inland Pacific Northwest Region - An 18-year retrospective study: 1991–2008. Journal of Avian Medicine and Surgery 24:279-287.

Steidl, R. J., and R.G. Anthony. 1996. Responses of bald eagles to human activity during the summer in Interior Alaska. Ecological Applications 6:482-491.

Steidl, R. J., K.D. Kozie, and R.G. Anthony. 1997. Reproductive Success of Bald Eagles in Interior Alaska. Journal of Wildlife Management 61:1313-1321.

Steidl, R. J., and R.G. Anthony. 2000. Experimental effects of human activity on breeding bald eagles. Ecological Applications 10:258-268.

Strom, S. M., J.A. Langenberg, N.K. Businga, and J.K. Batten. 2009. Lead exposure in Wisconsin birds. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI10.4080/ilsa.2009.0205.

Swenson, J.E., K.L. Alt, R.L. Eng. 1986. Ecology of bald eagles in the Greater Yellowstone ecosystem. Wildlife Monographs 95:3-46.

Wood, P.B., and M.W. Collopy 1993. Effects of egg removal on bald eagle productivity in Northern Florida. Journal of Wildlife Management 57:1-9.

CHAPTER 2. LEAD EXPOSURE IN FREE-FLYING BALD EAGLES IN IOWA

A paper to be submitted to Ecotoxicology

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ABSTRACT

In North America, the impacts of lead ammunition on free-flying Bald Eagles (*Haliaeetus leucocephalus*) and other raptors are a subject of considerable debate. We investigated temporal and spatial patterns in dietary lead exposure in wintering and nesting Bald Eagles in Iowa by collecting fecal samples from the base of roost and nest trees in 2012 and 2013. We documented detectable amounts of lead (>0.11 mg/kg) in 93% of feces. In the majority of samples, fecal lead levels were low and within the range of fecal lead levels found in other bird species inhabiting known non-lead contaminated sites and also similar to background environmental lead levels. Lead levels from nests sampled during the winter were higher than nests sampled in the spring (1.63 mg/kg vs. 1.09 mg/kg) and lead levels from non-Mississippi River nests were higher than Mississippi River nests (1.81 mg/kg vs. 0.90 mg/kg), but these differences were not statistically significant ($P > 0.05$). Lead levels in samples from nesting eagles were higher than samples from wintering eagles (1.37 mg/kg vs. 1.26 mg/kg), but these differences were not statistically significant ($P > 0.05$). We also found no statistical difference between fecal lead levels from nest sites on public versus private land (1.89 mg/kg vs. 0.98 mg/kg) and no correlation between lead exposure and land ownership, watershed, proximity to water, proximity to potential environmental sources of lead, or

number of deer harvested or lost due to wounding. Our results indicate that the majority of free-flying nesting and wintering Bald Eagles in Iowa experience low levels of lead exposure.

INTRODUCTION

In recent years, the debate on whether lead ammunition is linked to lead poisoning in the environment has placed much attention on the Bald Eagle (New York Times 2010, Sheridan 2012). Raptor rehabilitators throughout the U.S. continue to report cases of lead exposure in Bald Eagles and other raptors and have also noticed temporal correlations between the incidence of lead exposure and the timing of upland and big game hunting seasons (Kramer and Redig 1997, Harris and Sleeman 2007, Neumann 2009, Redig et al. 2009, Strom et al. 2009, Stauber et al. 2010, Neumann, personal communication). Environmentalists have inferred that lead exposure in Bald Eagles and other raptors may be attributable to the ingestion of lead ammunition (Craig et al. 1990, Clark and Scheuhammer 2003, Harradine et al. 2004, Fisher et al. 2006, Hunt et al. 2006, Saito 2009, Kelly et al. 2011, Nam et al. 2011, Bedrosian et al. 2012, Finkelstein et al 2012, Pagel et al. 2012). Despite that position, gun advocacy groups consider any discussion about the banning of lead ammunition an attack on gun rights (Kopel n.d., Smith n.d.). To date, the magnitude and timing of lead exposure in eagles and other wildlife is largely unresolved. Because of the complexity of this debate, lawmakers have yet to make an informed decision on the issue.

Lead is a poisonous substance to all animals, including humans. The impacts of lead exposure vary depending on the amount of lead an individual is exposed to and range from mild negative impacts to death (Eisler 1988, ATSDR 2007). As it accumulates, lead replaces other metals normally used for metabolism and increasingly disrupts the nervous, vascular,

renal, reproductive, and hematopoietic systems (Eisler 1988). As a consequence, an animal may experience diminished growth, stunted development, abnormal metabolism, difficulty learning, altered behavior, impaired reproduction, reduced survival (Eisler 1988). Spent lead shot, slugs, and bullets can remain inside unclaimed animal carcasses and accumulate at shooting ranges and frequently used hunting areas (Scheuhammer and Norris 1995, Thomas 1997, Sanborn 2002, Hunt et al. 2006, USGS 2008). The ingestion of spent lead ammunition, lead fragments embedded in food items, and lead fishing tackle have all been linked to morbidity and mortality in numerous species of birds including Bald Eagles (Locke 1982, Franson 1996, Kendall et al. 1996, Vyas 2000, Sanborn 2002, Clark and Scheuhammer 2003, Harradine et al. 2004, Fisher et al. 2006, Knopper 2006, Cade 2007, Craighead and Bedrosian 2008, Martin et al. 2008, Domenech and Langner 2009, Bedrosian et al. 2012). While the majority of ingested lead is excreted through regurgitation, defecation, or sequestration in feathers, some lead can be retained and accumulates in tissue (Platt 1976, Pattee et al. 1981, Leonzio and Massi 1989, Burger 1993, Pain et al. 1997, Sanderson 2002, Dauwe et al. 2003, Ek et al. 2004, Nam et al. 2004, Mateo et al. 2006, ATSDR 2007, Bergdahl and Skerfving 2008, Martinez-Haro et al. 2010, Route et al. 2011). Unlike waterfowl, raptors like Bald Eagles do not have a muscular gizzard which can erode lead fragments through the grinding action of grit, but their stomach pH is very low (pH 1-4) which makes lead very soluble (Clemens et al. 1975, Dieter and Finley 1978, Roscoe et al. 1979, Pain et al. 1997). When lead is not excreted, it is absorbed into the blood stream through the gastrointestinal tract. Dissolved lead is then deposited in soft tissues, such as the liver and kidneys, and ultimately accumulates in the skeleton, especially in areas of active bone formation (Scheuhammer 1987, Eisler 1988, Ek et al. 2004, Martin et al. 2008,

Martinez-Haro et al. 2010). Absorption and retention can vary greatly among individuals depending on age, sex, and diet (Pattee et al. 1981, Eisler 1988, Wayland et al. 1999).

One of the challenges to characterizing the magnitude of lead exposure in free-flying Bald Eagles is getting samples. While blood samples might be the most commonly-used biomarker to quantify lead exposure (Bergdahl and Skerfving 2008), the potential for negative impacts resulting from physically handling birds and climbing nest trees make blood sampling less than ideal when dealing with large birds in a large-scale field study (Richardson and Miller 1997, Rosenfield et al. 2007). A potentially informative alternative to blood sampling is to test fecal matter for lead because, similar to blood, it represents acute short-term dietary exposure. Also when dealing with large birds it can be easier to collect through non-invasive methods by collecting from the ground below nest trees. To the best of our knowledge, Bald Eagle fecal samples have not yet been used as a biomarker for lead exposure, although fecal lead levels have been successfully used to measure lead exposure in other species including: Blue and Great Tit, Great Blue Heron, Greylag Geese, Peregrine Falcon, Pied Flycatcher, Little Blue Herons, and Mallards (Fitzner et al. 1995, Eeva and Lehikoinen 1996, Spahn and Sherry 1999, Dauwe 2000, Sanderson 2002, Ek et al. 2004, Tiller et al. 2005, Mateo et al. 2006, Berglund et al. 2012, Martinez-Haro et al. 2010). Fecal lead levels have also been positively correlated with lead levels in the blood and liver in Greylag Geese and in humans (Mateo et al. 2006, Bergdahl and Skerfving 2008). Given the success of other studies in using feces as a biomarker, we chose to collect fecal matter from free-flying Bald Eagles for this study.

In Iowa, between January 2004 and December 2013, 59% of all Bald Eagles (164 out of 278) admitted to rehabilitation facilities had measurable amounts of lead in their blood

(Neumann et al. 2014). However, nothing is known about lead exposure in the free-flying population of Bald Eagles. The objective of this study was to characterize lead levels in the feces of nesting and wintering Bald Eagles in Iowa and identify temporal and spatial factors associated with elevated lead exposure. This study will provide an increased understanding of lead exposure in free-flying Bald Eagles in Iowa and help to further inform the future management and political decisions regarding Bald Eagles and lead ammunition in Iowa.

METHODS

Study Species

The Bald Eagle is a year-round resident in Iowa, although their numbers vary seasonally. There are an estimated 200-300 active Bald Eagle nests in the state (Shepherd and Nixon 2011, Shepherd 2013) and the number of wintering eagles is in the thousands. Bald Eagle numbers peak in Iowa between November and January, when migrants arrive from their breeding grounds in more northern states and Canada (Jackson et al. 1996). Bald Eagles nest in Iowa from late January through July, with incubation starting in late February. Eagles occur in the greatest concentrations wherever there is open water and an abundant food source (Kent and Dinsmore 1996, Dyar 2010). Bald Eagle nests are most heavily concentrated in the northeastern part of the state. Bald Eagles also nest along river corridors throughout the remainder of Iowa, but at lower densities, with the fewest nests occurring in western Iowa (Iowa DNR 2011, Iowa DNR 2012).

Study area

We randomly selected 110 Bald Eagle nests from a list of more than 200 nests classified as active by the Iowa Department of Natural Resources and the United States Fish and Wildlife Service in 2011 (Iowa DNR 2011). Nearly half of these nests were in the Upper

Mississippi River National Fish and Wildlife Refuge in northeastern Iowa, while the remaining nests were scattered throughout the rest of Iowa on both private and public lands. Nests sampled in 2012 were resampled in 2013 and any nests that were found abandoned, destroyed, or inaccessible in 2013 were replaced with randomly chosen backup nests. We opportunistically collected feces deposited by wintering Bald Eagles in central Iowa at 6 sites in 2012 and at 5 sites in 2013 (Figure 1).

Sample Stratification

Nest sampling was spatially and temporally stratified to enable us to test two specific hypotheses about lead exposure in Iowa. First, nests were spatially stratified into a Mississippi River group and a non-Mississippi River group. We hypothesized that fecal lead levels from Bald Eagles nesting away from the Mississippi River would be higher than fecal lead levels from Bald Eagles nesting near the Mississippi River. We expected that eagles nesting near the Mississippi River would more likely eat fish and waterfowl, while eagles nesting away from the Mississippi River would more likely scavenge on hunter-killed deer carcasses and gut piles that might contain lead fragments during the winter and early spring when interior lakes and rivers remained largely frozen. To stratify nests into two groups, a 7-km^2 buffer was placed around all nests assuming a home range size after Gerrard et al. (1992). Any buffered nests that overlapped the Mississippi River were included in the Mississippi River adjacent group. Any buffered nests that did not overlap the Mississippi River were placed in the non-Mississippi River group. We also stratified nests temporally by sampling each nest site once during winter and once during spring. We hypothesized that lead levels from samples collected in winter would be higher than in spring because nesting

eagles would have more access to hunter-killed deer carcasses and gut piles in the winter, shortly after the January deer hunting season, than they would later in the spring.

Fecal sampling of wintering and nesting Bald Eagles

Lead exposure in nesting and wintering Bald Eagles was quantified using fecal samples. All samples were collected from below wintering and nesting Bald Eagles using non-invasive collection techniques to reduce sampling-associated disturbance (Rosenfield et al. 2007). We conducted nest site sampling from 16 February to 29 March 2012 (winter) and from 4 May to 22 May 2012 (spring). In 2013, we conducted nest site sampling from 20 February to 28 March (winter) and from 1 May to 3 June (spring). For nesting eagles, the first fecal sampling visits occurred during the incubation period and the second sampling visits occurred when the Bald Eagle chicks were 3-9 weeks old. Nest sites were accessed on foot where possible and by boat where necessary on the Mississippi River. We spent ≤ 30 minutes within 100 m of each nest site. Fecal samples below a nest could be from 2-5 individuals depending on the time of the visit. During winter visits 2 adults were present at the nest incubating eggs, whereas during spring 2 adults were present along with 1-3 nestlings. Because of this, feces collected below each nest were mixed together and tested as one sample, with the resulting lead level being representative of the nest. We also opportunistically collected and tested individual fecal samples at 20 nest sites in 2013 to better understand the variation in lead levels among fecal samples at a single nest. The lead levels of individual fecal samples from these 20 nest sites were also averaged to represent the overall fecal lead level at each nest site.

In 2012 and 2013, we conducted fecal sampling at wintering roost sites between January and March in central Iowa. We opportunistically collected and tested feces deposited by individual wintering eagles where concentrations of eagles were accessible and available.

Environmental substrate sampling at Bald Eagle wintering roosts and nests

To better understand background lead levels at sampling sites, we collected substrate samples (soil, leaves, bark, snow, water) at nesting and wintering locations specifically focusing on substrates from which we collected feces. For example, if we collected feces from soil and leaves at a site, we also collected soil and leaf samples from the site.

Sample preparation and lead testing

All fecal samples were inspected under a dissecting microscope and cleaned of any substrate that did not pass through the bird (i.e., leaves, bark, twigs, and soil) to minimize background environmental contamination that might bias results. Processed feces and environmental substrate samples were transferred to the State Hygienic Laboratory (SHL) at the University of Iowa where lead levels were determined using inductively coupled plasma mass spectrometry (ICP-MS). The testing process involved an acid digestion using diluted nitric acid to break down any solid matter in the sample and allow any lead particles to go into solution. The solution was ionized by cooling it to 2°C and then pumping it into plasma where it was heated to 5,000-7,000°C. Any lead ions in the sample were counted by the mass spectrometer. The SHL typically reports lead levels below 0.94 mg/kg as <1.0 mg/kg, because that threshold represents their reporting limit, or ability to discern the difference between background noise and a valid signal attributable to the analyte of interest. Lead levels from 0.95 - 1 mg/kg are reported as 1 mg/kg, and lead levels >1.0 mg/kg are reported as actual values. For statistical analyses, we used the instrument read-out values for all

samples measuring <1.0 mg/kg acknowledging that there might be some error at these small concentrations. Lead levels from 0.0 to 0.1 mg/kg were considered effectively zero based on the SHL testing standards.

Data Analyses

Fecal lead levels were compared a) between samples collected at nest sites during the winter and spring, b) between samples collected at non-Mississippi River and Mississippi River nest sites, and c) between samples collected from wintering and nesting Bald Eagles using an ANOVA ($\alpha = 0.05$) in R-Studio (RStudio 2013). We also calculated means, 95% confidence limits, medians, and ranges for each group and for 20 individually collected samples at nests (10 winter, 10 spring) in 2013. It was not economically feasible to test all environmental substrates collected, so we tested 1-2 substrates from any nest site with fecal lead levels >1.0 mg/kg and one substrate from a random selection of nest sites that had fecal lead levels <1.0 mg/kg. We calculated means, 95% confidence limits, medians, and ranges for each substrate type sampled (soil, leaves, bark, snow), and compared lead levels (by substrate type) for nest sites and wintering roost sites that had fecal lead levels of >1.0 mg/kg versus <1.0 mg/kg using an ANOVA.

Roadways, mining sites, industrial areas, older houses, old orchards, power plants, incinerators, landfills, hazardous waste sites and shooting ranges are the most common places to find lead-laden soil (Cao et al. 2003, ATSDR 2007). To evaluate the relationships between fecal lead levels and landscape features that might influence lead exposure, we identified and mapped potential sources of lead in the environment including roads, railroads, contaminated sites, ethanol and biodiesel facilities, municipal landfills, and municipal gas and electric utilities, as well as other potentially important variables such as major rivers and lakes,

watersheds, and land ownership in Iowa. ArcMap (ESRI 2013) was used in conjunction with data obtained from the Natural Resources Geographic Information Systems Library (NRGIS 2013) to create these maps.

Four watersheds (Missouri-Little Sioux, Missouri-Nishnabotna, Grand, and Chariton rivers) were combined together because they each included only one nest with four samples each. The fecal lead levels at nest sites within the resulting five watershed basins were compared for each season (winter and spring). Additionally, fecal lead levels at nest sites were compared between seasons within each watershed basin. Fecal lead levels were compared between nest sites on public and private land and between nest sites in different watershed basins using an ANOVA. Next, we examined fecal lead levels at nest sites as a function of their proximity to water and potential environmental sources of lead. We calculated the distance, in meters, between each sampled nest site and the nearest: roads, railroads, major rivers and lakes, contaminated sites, ethanol and biodiesel facilities, municipal landfills, and municipal gas and electric utilities. We used linear regressions to determine whether fecal lead levels were statistically significantly related to the proximity of nest sites to any of these features.

To evaluate relationships between lead exposure at nest sites and the number of deer harvested, we calculated mean fecal lead levels for 13 counties where we sampled at least 2 nests (Allamakee, Boone, Buchanan, Butler, Clayton, Dallas, Floyd, Jackson, Linn, Marion, Polk, Story, and Winneshiek counties) and then compared those means to deer harvest data (Iowa DNR 2013). We used deer harvest data from 2011 and 2012, because we hypothesized that the fecal lead levels of nesting and wintering Bald Eagles in 2012 and 2013, if

influenced by lead ammunition, would be most influenced by the harvest levels of the preceding fall deer-hunting season.

We used linear regressions to determine whether fecal lead levels were statistically related to the number of antlered deer harvested, total number of deer harvested, and the estimated number of unrecovered carcasses (estimated at 10%) for 2011 and 2012. We compared 2011 deer data to 2012 fecal lead levels, 2012 deer data to 2013 fecal lead levels, and deer data (averaged for 2011 and 2012) to fecal lead levels (averaged for 2012 and 2013). We also used linear regressions to compare winter fecal lead levels and spring fecal lead levels (averaged for 2012 and 2013) to deer data (averaged for 2011 and 2012).

The fecal sampling procedures at nest and roost sites described above were conducted with permission from the United States Fish and Wildlife Service (permit #MB52842A-0) and the state of Iowa (permit #SC-871 and #SC-872).

RESULTS

Fecal samples were collected from below 110 nests in 2012 and 107 nests in 2013 (Figure 1). Of the 110 nests visited in winter 2012, fecal samples from 107 nests were tested for lead. Fecal samples collected below three nests were not tested because their quantities were too small due to high water levels at each nest site. In spring 2012, fecal samples from 102 nests were sent for testing. The eight remaining nest sites had little to no feces present and were considered inactive due to an unkempt appearance of the nest and the absence of adults or nestlings. For nest sites that had fecal lead levels of >1.0 mg/kg, we tested 1-2 environmental substrates (n=92). One substrate sample (typically soil) was also tested for 62 randomly selected nest sites that had fecal lead levels of <1.0 mg/kg. We also tested 83 fecal

samples and 8 environmental substrates from wintering eagle sites. In total, we tested 454 fecal and substrate samples during 2012 (Table 1).

In 2013, of 107 nests sampled in the winter, 85 nests were resampled in the spring. The remaining 22 nests were both inactive and not sampled (13), active but we could not find any feces because of high river water (2), or inaccessible because of high river water (7). We tested 1-2 environmental substrates from nest sites with fecal lead levels of >1.0 mg/kg (n=79) and one substrate from nests that had fecal lead levels of <1.0 mg/kg (n=51). We also tested 86 fecal samples and 8 environmental substrates from wintering eagle sites in 2013. In total, we tested 554 fecal and substrate samples during 2013 (Table 1).

In 2013, we also tested 158 individual samples collected from 19 nest sites (one nest site was sampled in both winter and spring) to better understand intra-nest variation in fecal lead levels. We tested 70 individual samples (from 10 nest sites) during winter and 79 individual samples (from 10 nest sites) in the spring. We then tested an additional 9 samples (from 9 out of 10 spring nests), by mixing together portions of fecal matter from the individual spring samples (weighing >0.5g) to get a sample representative of each nest (mimicking our sampling approach for the majority of nest sites). We then compared these mixed samples to the individual fecal samples to better understand the variation in lead levels among fecal samples at a single nest.

We documented measurable amounts of lead in 93% of the feces collected from nesting and wintering Bald Eagles in 2012 and 2013 (Figures 2-4). Lead levels from wintering eagles were not statistically different between years (P >0.05). Similarly, fecal lead levels from nest sites were not statistically different in the same season between years (P

>0.05). We concluded that combining both years of nest site data as well as both years of wintering eagle data together was justified for further analysis.

Lead levels from nesting eagles were higher in the winter than in the spring (1.63 mg/kg vs. 1.09 mg/kg; Figure 2) and lead levels were higher at non-Mississippi River nests than Mississippi River nests (1.81 mg/kg vs. 0.90 mg/kg; Figure 3), but these differences were not statistically significant ($P > 0.05$). The highest fecal lead level for nesting samples was 170 mg/kg, but this was an extreme case. In addition, lead levels for samples from nesting eagles were higher than for samples from wintering eagles (1.37 mg/kg vs. 1.26 mg/kg; Figure 4), but these differences were not statistically significant ($P >0.05$). Nest sites where individual samples were collected and tested in 2013 (n=20) had means ranging from 0 mg/kg to 1.29 mg/kg (Figures 5-6). Furthermore, a comparison of fecal lead levels in mixed samples (samples representing a nest consisting of a mixture of individual samples) versus the fecal lead means of individual samples collected in spring 2013 exhibited little difference in mean fecal lead level per nest (Table 4).

We documented measurable amounts of lead in 79% of environmental substrates collected from below nests and winter roosts in Iowa in 2012 and 2013 (Table 5). Soil samples had the highest mean lead level (7.05 mg/kg, 95% CL [6.23, 7.87]), followed by bark (1.55 mg/kg, 95% CL [1.16, 1.95]), leaves (0.56 mg/kg, 95% CL [0.36, 0.75]), and snow (0 mg/kg, 95% CL [0, 0]). Soil lead levels were higher at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels <1.0 mg/kg (7.41 mg/kg vs. 6.97 mg/kg), but these differences were not statistically significant ($P > 0.05$). Leaf lead levels were also higher at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels < 1.0 mg/kg (0.61 mg/kg vs. 0.31 mg/kg), but

these differences were also not statistically significant ($P > 0.05$). We found bark lead levels to be lower at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels <1.0 mg/kg (1.28 mg/kg vs. 1.96 mg/kg), but these differences were not statistically significant ($P > 0.05$). All snow samples had lead levels <0.1 mg/kg.

Nesting eagles on private lands had higher mean lead levels than those on public lands (1.89 mg/kg vs. 0.98 mg/kg), but these values were not statistically significant ($P > 0.05$). In addition, there was no statistical difference ($P > 0.05$) in lead levels among watersheds when comparing within the same season (Figures 7-8). Although nesting eagles in the Des Moines River basin had a higher overall mean in the winter, the variance in that basin was large (mean = 6.82 mg/kg, 95% CL [-5.59, 19.22]). Similarly, there was no statistical difference ($P > 0.05$) when comparing seasons within each individual watershed, except for the combined Missouri-Little Sioux-Nishnabotna-Grand-Chariton River basin, where nesting eagles had statistically higher fecal lead levels ($P = 0.04$) in spring (mean = 1.73) compared to winter (mean = 0.91). Linear regressions testing the relationship between fecal lead levels in nesting eagles and proximity to nearest roads, railroads, major rivers and lakes, contaminated sites, ethanol and biodiesel facilities, municipal landfills, and municipal gas and electric utilities were not statistically significant. Furthermore, linear regressions testing the relationship between mean nesting fecal lead levels in 13 counties and antlered deer harvest, total deer harvest, and estimate of unrecovered carcasses for 2011 and 2012 were not statistically significant.

DISCUSSION

We found various lead levels in the feces of nesting and wintering Bald Eagles in Iowa. Fecal lead levels in the majority of samples were low and within the range of fecal lead

levels documented in other bird species inhabiting non-lead contaminated sites (Fitzner et al. 1995, Dauwe et al. 2000, Ek et al. 2004, Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010). In Belgium for example, Dauwe et al. (2000) found that Great (*Parus major*) and Blue Tit (*Parus caeruleus*) had significantly lower mean fecal lead levels at reference sites (2.34 mg/kg and 5.54 mg/kg, respectively) compared to sites near a metallurgic facility (80.4 mg/kg and 124.8 mg/kg, respectively). Similarly, Berglund et al. (2010) found that Pied Flycatchers (*Ficedula hypoleuca*) in Sweden had significantly lower mean fecal lead levels at reference sites (0.5 - 0.7 mg/kg) compared to sites near a lead mine and enrichment plant (23 - 40 mg/kg). Fitzner et al. (1995) found higher fecal lead levels in the majority of Great Blue Heron colonies located in or near areas of increased urbanization or industrial development in western and eastern Washington (0.37 - 10.45 mg/kg) compared to colonies located in agricultural or range lands and more isolated from human and industrial development (0.20 - 7.36 mg/kg). Tiller et al. (2005) also found that fecal lead levels (1.0, 1.3, 3.5 mg/kg) of Great Blue Heron (*Ardea Herodias*) in Washington were similar to lead levels in the river sediment. Ek et al. (2004) found a similarly low mean fecal lead level of 1.5 mg/kg in wild Peregrine Falcons (*Falco peregrinus*) in rural Sweden.

We found 16 (2.8%) nesting and wintering fecal samples with lead levels greater than 5.0 mg/kg (9 between 5.1-10.0 mg/kg, 4 between 10.1-20.0 mg/kg, 2 between 20.1-25.0 mg/kg, and one measuring 170.0 mg/kg) out of 570 total samples collected from nesting and wintering Bald Eagles. The highest fecal lead level of 170 mg/kg, collected during the winter at a non-Mississippi River nest, may be the result of exposure to lead ammunition, since it was significantly higher than any substrate samples collected from the site (soil = 9.9 mg/kg and leaves = 0.32 mg/kg). However, when the nest was revisted in the spring, we

documented a fecal lead level of 0.81 mg/kg and observed that the nest was still active with one adult and at least one nestling present. This observation indicates that Bald Eagles have the ability to excrete high levels of lead in their feces. The next two highest nesting fecal lead levels (22 mg/kg and 14 mg/kg) were both collected during the spring, when both nests were active with nestlings. The next two highest nesting fecal lead levels (11 mg/kg and 8 mg/kg) were both collected during the winter and both nests were active during our spring visit and had lower lead levels (0.59 mg/kg and 0 mg/kg, respectively). While we did not follow these nests to fledging our observations of active nests with nestlings present in the spring following high lead levels in the winter suggests that high lead levels do not neccesarily negatively affect reproductive success. Tiller et al. (2005) reported that despite the proximity of Great Blue Heron colonies to nine retired plutonium production reactors (a past contributor of heavy metals into the environment), these colonies had among the highest reproductive health in the continental U.S., suggesting that low levels of lead in feces did not negatively affect nesting success.

In 1997, Kramer and Redig retroactively reviewed 138 cases of lead-exposed Bald and Golden eagles admitted to The Raptor Center at the University of Minnesota to identify the relationships between blood lead levels and clinical symptoms. They reported that during 16 years, blood lead levels >1.2 mg/kg were always fatal, while levels between 0.61 and 1.2 mg/kg represented clinical and treatable lead poisoning, and levels between 0.2 and 0.6 mg/kg represented subclinical lead exposure. We found that fecal lead levels are positively correlated with blood lead levels in Bald Eagles admitted to rehabilitation centers, but it is not a 1:1 relationship (Lead exposure in free-flying versus rehabilitation bald eagles; see

Chapter 3). Therefore, clinical symptoms based on fecal lead levels cannot be inferred from the Kramer and Redig (1997) criteria.

We hypothesized that there would be spatial differences in lead exposure between the feces of Bald Eagles nesting on the Mississippi River and the feces of Bald Eagles nesting away from the Mississippi River. However, there was no statistical difference between the groups, indicating there is no strong support for higher levels of lead exposure at non-Mississippi River nests due to greater access to deer carcasses and gut piles. We also hypothesized that there would be temporal differences in lead exposure between feces collected in the winter compared to feces collected in the spring. However, we found no statistical difference between the groups. Therefore, there is no strong support for higher levels of lead exposure in feces collected in closer temporal proximity to the deer-hunting season.

We found various lead levels in the substrates collected from below Bald Eagle nest and roost sites in Iowa, yet the ranges were consistent with lead levels typically found across the US (ATSDR 2007, EPA 1980). Mean soil lead levels were higher than fecal lead levels in the majority of samples from nesting and wintering Bald Eagles, while mean bark and leaf lead levels were similar to the majority of fecal lead levels. The lack of a significant statistical difference between substrate lead levels at sites with fecal lead levels >1.0 mg/kg and those with fecal lead levels <1.0 mg/kg suggests that the variation found in fecal lead levels was likely due to the variation in ingestion of lead by Bald Eagles rather than the contamination of our samples by substrates post-excretion. If fecal lead levels in our study were influenced by their proximity to potential environmental sources of lead, we would have expected to observe that as distance of a nest site from these sources increased that fecal

lead levels would decline, but this was not the case. The lack of statistical difference between fecal lead levels between public and private lands, watersheds, and the lack of statistically significant relationships between fecal lead levels and potential environmental sources of lead, suggests that environmental sources of lead do not appear to be strongly affecting lead exposure in Bald Eagles.

Overall, the magnitude of lead exposure in nesting and wintering Bald Eagles in Iowa was low in the majority of cases and similar to lead levels typically found in the environment. The levels of lead exposure we documented in Bald Eagles were also similar to other studies where birds inhabited non-lead contaminated sites. Our results indicate that 1) the majority of free-flying nesting and wintering Bald Eagles in Iowa experience low levels of lead exposure, and 2) that concerns about widespread exposure of Bald Eagles to lethal lead levels in Iowa are not well supported from this study.

LITERATURE CITED

Agency for Toxic Substances and Disease Registry (ATSDR). 2007. Public health statement for lead, summary of the toxicological profile for lead. U.S. Department of Health and Human Services, Public Health Service. Atlanta, GA.

Bedrosian, B., D. Craighead, and R. Crandall. 2012. Lead exposure in bald eagles from big game hunting, the continental implications and successful mitigation efforts. PLoS ONE 7(12): e51978. doi:10.1371/journal.pone.0051978.

Bergdahl. I.A., and S. Skerfving. 2008. Biomonitoring of lead exposure-alternatives to blood. Journal of Toxicology and Environmental Health, Part A 71:1235-1243.

Berglund, A.M., P.K. Ingvarsson, H. Danielsson, and N.E.I. Nyholm. 2010. Lead exposure and biological effects in pied flycatchers (*Ficedula hypoleuca*) before and after the closure of a lead mine in northern Sweden. Environmental Pollution 158:1368-1375.

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. Reviews in Environmental Toxicology 5:203-311.

Cade, T.J. 2007. Exposure of California condors to lead from spent ammunition. Journal of Wildlife Management 71:2125-2133.

Cao, X., L.Q. Ma, M. Chen, D.W. Hardison, Jr., and W.G. Harris. 2003. Weathering of lead bullets and their environmental effects at outdoor shooting ranges. Journal of Environmental Quality 32:526-534.

Clark, A.J., and A.M. Scheuhammer. 2003. Lead poisoning in upland-foraging birds of prey in Canada. Ecotoxicology 12:23-30.

Clemens, E.T., L. Krook, A.L. Aronson, and C.E. Stevens. 1975. Pathogenesis of lead shot poisoning in the mallard duck. The Cornell Veterinarian 65:248-285.

Craig, T. H., J. W. Connelly, E. H. Craig, and T. L., Parker. 1990. Lead concentrations in golden and bald eagles. The Wilson Bulletin 102:130-133.

Craighead, D., and B. Bedrosian. 2008. Blood lead levels of common ravens with access to big-game offal. Journal of Wildlife Management 72:240-245.

Dauwe, T., L. Bervoets, R. Blust, R. Pinxten, and M. Eens. 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Archives of Environmental Contamination and Toxicology 39:541-546.

Dauwe, T., L. Bervoets, R. Pinxten, R. Blust, and M. Eens. 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. Environmental Pollution 124:429-436.

Dieter, M. P. and M. T. Finley. 1978. Erythrocyte delta-aminolevulinic acid dehydratase activity in mallard ducks: duration of inhibition after lead dosage. Journal of Wildlife Management 42(3):621-624.

Domenech, R., and H. Langner. 2009. Blood-lead levels of fall migrant golden eagles in west-central Montana. Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA, DOI 10.4080/ilsa.2009.0210.

Dyar, J. 2010. Bald Eagles in Iowa. Iowa Department of Natural Resources

Eeva, T., and E. Lehikoinen. 1996. Growth and mortality of nestling great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 102:312-323.

Eisler, R. 1988. Lead hazards to fish, wildlife, and invertebrates: a synoptic review. United States Fish and Wildlife Service, report no. 85, Washington, D.C., USA.

Ek, K.H., G.M. Morrison, P. Lindberg, and S. Rauch. 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Archives of Environmental Contamination and Toxicology 47:259-269.

EPA. 1980. Ambient water quality criteria for lead. U.S. Environmental Protection Agency Report 440/5-80-057.

EPA. 2007. Method 6020A: Inductively coupled plasma-mass spectrometry. Revision 1, February 2007 <www.epa.gov/osw/hazard/testmethods/sw846/pdfs/6020a.pdf>. Accessed 30 April 2012.

ESRI 2013. Arc GIS Desktop: Release 10.1. Redlands, CA: Environmental Systems Research Institute.

Finkelstein, M.E., D.F. Doak, D. George, J. Burnett, J. Brandt, M. Church, J. Grantham, and D.R. Smith. 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. PNAS 109:11449-11454.

Fisher, I.J., D.J. Pain, and V.G. Thomas. 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biological Conservation 131:421-432.

Fitzner, R.E., R.H. Gray, and W.T. Hinds. 1995. Heavy metal concentrations in great blue heron castings in Washington state: a technique for monitoring regional and global trends in environmental contaminants. Bulletin of Environmental Contamination and Toxicology 55:398-403.

Franson, J.C. 1996. Interpretation of tissue lead residues in birds other than waterfowl. Pages 265-279 in W. N. Beyer, G. H. Heinz, and A. W. Redwood-Norwood (Eds.). Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Lewis Publishers, New York, USA.

Gerrard, J., A.R. Harmata, and P.N. Gerrard. 1992. Home range and activity of a pair of bald eagles breeding in northern Saskatchewan. Journal of Raptor Research 26:229-234.

Harradine, J. 2004. Spent lead shot and wildlife exposure and risks. Pages 119-130 in Scheinosst, A. (ed.), Proceedings of the World Symposium on Lead Ammunition. World Forum on the Future of Sport Shooting Activities. September 9-10, 2004. Rome, Italy.

Harris, M. C. and J. M. Sleeman. 2007. Morbidity and mortality of Bald Eagles (*Haliaeetus leucocephalus*) and Peregrine Falcons (*Falco peregrinus*) admitted to the wildlife center of Virginia, 1993–2003. Journal of Zoo and Wildlife Medicine 38:62-66.

Hunt, W. G., W. Burnham, C. N. Parish, K. K. Burnham, B. Mutch, and J. L. Oaks. 2006. Bullet fragments in deer remains: implications for lead exposure in avian scavengers. Wildlife Society Bulletin 34:167-170.

Iowa Department of Natural Resources (Iowa DNR). 2011. Unpublished bald eagle nesting data.

Iowa Department of Natural Resources (Iowa DNR). 2012. Unpublished bald eagle nesting data.

Iowa Department of Natural Resources (Iowa DNR). 2013. Unpublished deer harvest data.

Jackson, L.S, C.A. Thompson, J.J. Dinsmore, B.L. Ehresman, J. Fleckenstein, R. Cecil, L.M. Hemesath, S.J. Dinsmore. 1996. The Iowa Breeding Bird Atlas. University of Iowa Press. Iowa City, IA. 484 p.

Kelly, T. R., P. H. Bloom, S. G. Torres, Y. Z. Hernandez, R. H. Poppenga, W. M. Boyce, and C. K. Johnson. 2011. Impact of the California lead ammunition ban on reducing lead exposure in golden eagles and turkey vultures. Plos One 6: e17656.

Kendall, R. J., T. E. Lacher, Jr., C. Bunck, B. Daniel, C. Driver, C. E. Grue, F. Leighton, W. Stansley, P.G. Watanabe, and M. Whitworth. 1996. An ecological risk assessment of lead shot exposure in non-waterfowl avian species: upland game birds and raptors. Environmental Toxicology and Chemistry 15:4-20.

Kent, T.H. and J.J. Dinsmore. 1996. Birds in Iowa. Self published. Iowa City and Ames, IA. 391 p.

Knopper, L.D., P. Mineau, A.M. Scheuhammer, D.E. Bond, and D.T. McKinnon. 2006. Carcasses of shot Richardson's ground squirrels may pose lead hazards to scavenging hawks. Journal of Wildlife Management 70:295-299.

Kopel, D. n.d. The plan to get the lead out. National Rifle Association Publications <http://www.nrapublications.org/index.php/8991/the-plan-to-get-the-lead-out/>. Accessed 30 April 2012.

Kramer, J. L., and P. T. Redig. 1997. Sixteen years of lead poisoning in eagles, 1980–95: an epizootiologic view. Journal of Raptor Research 31:327-332.

Leonzio, C., and A. Massi. 1989. Metal biomonitoring in bird eggs: a critical experiment. Bulletin of Environmental Contamination and Toxicology 43:402-406.

Locke, L. N., S. M. Kerr, and D. Zoromski. 1982. Lead poisoning in common loons (*Gavia immer).* Avian Diseases 26:392-396.

Martin, P.A., D. Campbell, K. Hughes, and T. McDaniel. 2008. Lead in the tissues of terrestrial raptors in southern Ontario, Canada, 1995-2001. Science of the Total Environment 391:96-103.

Martinez-Haro, M., M.A. Taggart, and R. Mateo. 2010. Pb-Al relationships in waterfowl feces discriminate between sources of Pb exposure. Environmental Pollution 158:2485-2489.

Mateo, R., M.A. Taggart, A.J. Green, C. Cristofol, A. Ramis, H. Lefranc, J. Figuerola, and A.A. Meharg. 2006. Altered porphyrin excretion and histopathology of Greylag geese (*Anser anser*) exposed to soil contaminated with lead and arsenic in the Guadalquivir Marshes, southwestern Spain. Environmental Toxicology and Chemistry 25:203-212.

Morrissey, C.A., L.I. Bendell-Young, and J.E. Elliot. 2005. Assessing trace-metal exposure to American Dippers in mountain streams of southwestern British Columbia, Canada. Environmental Toxicology and Chemistry 24:836-845.

Nam, D.H., D.P. Lee, and T.H. Koo. 2004. Monitoring for lead pollution using feathers of feral pigeons (*Columba livia*) from Korea. Environmental Monitoring and Assessment 95:13-22.

Nam DH, J. Rutkiewicz, N. Basu. 2011. Multiple metals exposure and neurotoxic risk in bald eagles (*Haliaeetus leucocephalus*) from two Great Lakes states. Environmental Toxicology and Chemistry 31:623-631.

New York Times. 2010. The bullet that keeps on killing, editorial, Sept. 13, 2010.

Neumann, K. 2009. Bald eagle lead poisoning in winter. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0119.

Neumann, K. 2014. Personal communication.

Natural Resources Geographic Information Systems Library (NRGIS) 2013. <http://www.igsb.uiowa.edu/nrgislibx/>. Accessed 4 September 2012.

Pain, D.J., C. Bavoux, and G. Burnleau. 1997. Seasonal blood lead concentrations in Marsh Harriers (*Circus aeruginosus*) from Charente-Maritime, France: relationship with the hunting season. Biological Conservation 81:1-7.

Pagel, J.E., P.B. Sharpe, D.K. Garcelon, A.E. Little, S.K. Taylor, K.R. Faulkner, and C.S. Gorbics. 2012. Exposure of bald eagles to lead on the Northern Channel Islands, California. Journal of Raptor Research 46(2): 168-176.

Pattee, O. H., S. N. Wiemeyer, B. M. Mulhern, L. Sileo, and J. W. Carpenter. 1981. Experimental lead-shot poisoning in bald eagles. Journal of Wildlife Management 45:806-810.

Platt, J.B. 1976. Bald Eagles wintering in the Utah desert. American Birds 30:783-788.

RStudio. 2013. RStudio: Integrated development environment for R. Boston, MA. Version 0.97.236, http://www.rstudio.org/

Redig, P. T., D. R. Smith, and L. Cruz-Martinez. 2009. Potential sources of lead exposure for Bald Eagles: A retrospective study. Extended abstract in R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0208.

Richardson, C. T., and C. K. Miller. 1997. Recommendations for protecting raptors from human disturbance: a review. Wildlife Society Bulletin 25:634-638.

Roscoe, D.E., S.W. Nielsen, A.A. Lamola, and D. Zuckerman. 1979. A simple, quantitative test for erythrocytic protoporphyrin in lead-poisoned ducks. Journal of Wildlife Diseases 15:127-136.

Rosenfield, R. N., J. W. Grier, and R. W. Fyfe. 2007. Reducing management and research disturbance. Pages 351-364 in Raptor Research and Management Techniques. Hancock House Publishers. Surrey, Canada.

Route, B., P. Rasmussen, R. Key, M. Meyer, and M. Martell. 2011. Spatial patterns of persistent contaminants in bald eagle nestlings at three national parks in the Upper Midwest. National Park Service, National Resources Technical Report NPS/GLKN/NRTR - 2011/431.

Saito, K. 2009. Poisoning of Steller's sea-eagle (*Haliaeetus pelagicus*) and white-tailed eagle (*Haliaeetus albicilla*) caused by the ingestion of lead bullets and slugs, in Hokkaido Japan. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0304.

Sanborn, W. 2002. Lead poisoning of North American wildlife from lead shot and lead fishing tackle. HawkWatch International, Inc. Salt Lake City, UT.

Sanderson, G.C. 2002. Effects of diet and soil on the toxicity of lead in mallards. Ecotoxicology 11:11-17.

Scheuhammer A.M. 1987. The chronic toxicity of aluminium, cadmium, mercury, and lead in birds: A review. Environmental Pollution 46:263–295.

Scheuhammer, A.M., and S.L. Norris. 1995. A review of the environmental impacts of lead shotshell ammunition and lead fishing weights in Canada. Occasional Paper Number 88, Canadian Wildlife Service. National Wildlife Research Centre, Hull, Quebec. 56 p.

Shepherd, S. and B. Nixon. 2011. Iowa's 2011 midwinter bald eagle survey report. Iowa Department of Natural Resources.

Shepherd, S. 2013. Bald eagle (*Haliaeetus leucocephalus*) nesting in Iowa 2013. Iowa Department of Natural Resources. <http://www.iowadnr.gov/Portals/idnr/uploads/wildlife/research/BENestSummary_2012.pdf>. Accessed 28 January 2014.

Sheridan, K. 2012. Bald eagle in crosshairs of US fight over lead bullets, editorial, Associated Free Press. April 6, 2012. <http://www.google.com/hostednews/afp/article/ALeqM5iLMsEXWCoy78F_AZc0aDOfGpruHw?docId=CNG.1862d6191b237577b6f5f5b8cdfd5d8e.f1>. Accessed 30 May 2012.

Smith, B. n.d. EPA leaves lead alone. National Rifle Association Publications. <http://www.nrapublications.org/index.php/9290/epa-leaves-lead-alone/>. Accessed 28 January 2014.

Spahn S.A. and T.W. Sherry. 1999. Cadmium and lead exposure associated with reduced growth rates, poorer fledgling success of little blue heron chicks (*Egretta caerula*) in South Louisiana Wetlands. Archives of Environmental Contamination and Toxicology 37:377–384.

Stauber, E., N. Finch, P. A. Talcott, and J. M. Gay. 2010. Lead poisoning of bald (*Haliaeetus leucocephalus*) and golden (*Aquila chrysaetos*) eagles in the U.S. Inland Pacific Northwest Region—An 18-year retrospective study: 1991–2008. Journal of Avian Medicine and Surgery 24:279-287.

Strom, S. M., J.A. Langenberg, N.K. Businga, and J.K. Batten. 2009. Lead exposure in Wisconsin birds. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI10.4080/ilsa.2009.0205.

Thomas, V.G. 1997. The environmental and ethical implications of lead shot contamination of rural lands in North America. Journal of Agricultural and Environmental Ethics 10:41-54.

Tiller, B.L., J.D. Marco, and W.H. Rickard. 2005. Metal concentrations, foraging distances, and fledging success of Great Blue Herons nesting along the Hanford Reach of the Columbia River. Environmental Monitoring and Assessment 104:71-79.

United States Geological Survey (USGS). 2008. Lead shot and sinkers: weighty implications for fish and wildlife health. <http://www.usgs.gov/newsroom/article.asp?ID=1972#.T8aFoplYvDk>. Accessed 30 May 2012.

United States Fish and Wildlife Service (USFWS). 2007. Bald Eagle fact sheet: natural history, ecology, and history of recovery.

<http://www.fws.gov/midwest/eagle/recovery/biologue.html>. Accessed 18 February 2014.

Vyas, N.B., J.W. Spann, G.H. Heinz, W.N. Beyer, J.A. Jaquette, and J.M. Mengelkoch. 2000. Lead poisoning of passerines at a trap and skeet range. Environmental Pollution 107:159-166.

Wayland, M., E. Neugebauer, and T. Bollinger. 1999. Concentrations of lead in liver, kidney, and bone of Bald and Golden Eagles. Archives of Environmental Contamination and Toxicology 37: 267-272.

TABLES

Table 1. Summary of the number of fecal and substrate samples collected from the ground below nesting and wintering sites in Iowa during 2012 and 2013. All samples were tested for lead at the University of Iowa's State Hygienic Lab.

Sample Type	Source	2012	2013
Feces	Winter Nest	107	167
Feces	Spring Nest	102	163
Substrate	Nest Fecal lead >1.0	92	79
Substrate	Nest Fecal lead <1.0	62	51
Feces	Wintering	83	86
Substrate	Wintering	8	8
TOTAL		454	554

Table 2. Summary of fecal lead statistics from nesting Bald Eagles in Iowa in 2012 and 2013. Data from both years were combined together, because there were no statistical differences between years. Count, means, 95% confidence limits, medians, and ranges are presented below along with ANOVA results ($\alpha = 0.05$) comparing lead levels between the following groups: winter vs. spring nests and Mississippi River vs. non-Mississippi River nests. Individual samples (n=158 in 2013) were averaged to represent the 20 nests where samples were tested individually rather than combined into a single sample for testing. (Pb = lead, mg/kg = milligram/kilogram)

	Winter	Spring	Mississippi River	non-Mississippi River
# of samples	214	187	193	208
Pb Mean (mg/kg)	1.63	1.09	0.90	1.81
Confidence Limits (95%)	0.06, 3.19	0.8, 1.37	0.72, 1.09	0.19, 3.43
Pb Median (mg/kg)	0.48	0.73	0.59	0.55
Pb Range (mg/kg)	0 to 170	0 to 22	0 to 11	0 to 170
ANOVA	$F_{(1, 399)} = 0.39$ $P = 0.53$		$F_{(1, 399)} = 1.12$ $P = 0.29$	

Table 3. Summary of fecal lead statistics from wintering and nesting Bald Eagles in Iowa in 2012 and 2013. Data from both years were combined together because there were no statistical differences between years. Counts, means, 95% confidence limits, medians, and ranges are presented below with ANOVA results ($\alpha = 0.05$) comparing wintering vs. nesting eagles. Individual samples (n=158 in 2013) were averaged to represent the 20 nests where samples were tested individually rather than combined into a single sample for testing. (Pb = lead, mg/kg = milligram/kilogram)

	Wintering	Nesting
# of samples	169	401
Pb Mean (mg/kg)	1.26	1.37
Confidence Limits (95%)	0.80, 1.71	0.53, 2.22
Pb Median (mg/kg)	0.36	0.57
Pb Range (mg/kg)	0 to 25	0 to 170
ANOVA	$F_{(1, 568)} = 0.03$ $P = 0.86$	

Table 4. Summary of fecal lead levels (in milligram/kilogram) in individual samples (n=88) collected and tested from below 9 Bald Eagle nests in spring 2013 in Iowa comparing mixed samples (n=9) to samples tested separately and averaged (n=79) and the difference between them.

Nest ID	535	159	566	Maria's	270	460	Phil's	69	470
Mixed samples	0.29	0.29	0.27	0.22	0.54	0.53	0.59	0.58	1.20
Separate samples (95% CL)	0.27 (0, 0.77)	0.24 (0, 0.69)	0.71 (0,4.39)	0.54 (0, 2.32)	0.70 (0.36, 1.03)	0.60 (0.37, 0.83)	0.82 (0.36, 1.27)	0.56 (0.29, 0.84)	1.15 (0.56, 1.74)
Difference	0.02	0.05	0.44	0.32	0.16	0.07	0.23	0.02	0.05

Table 5. Summary of substrate lead statistics from nesting and wintering Bald Eagles in Iowa in 2012 and 2013. Count, means, 95% confidence limits, medians, and ranges are presented below along with ANOVA results ($\alpha = 0.05$) comparing lead levels by substrate type between nests and roost sites that had fecal lead levels of >1.0 mg/kg versus <1.0 mg/kg. (Pb = lead, mg/kg = milligram/kilogram)

	Soil <1.0 mg/kg	Soil >1.0 mg/kg	Leaves <1.0 mg/kg	Leaves >1.0 mg/kg	Bark <1.0 mg/kg	Bark >1.0 mg/kg	Snow <1.0 mg/kg	Snow >1.0 mg/kg
# of samples	56	48	34	83	28	42	6	3
Pb Mean (mg/kg)	6.97	7.41	0.31	0.61	1.96	1.28	0	0
CL(95%)	6.12, 7.82	6.07, 8.75	0.13, 0.49	0.37, 0.85	1.28, 2.63	0.8, 1.75	0, 0	0, 0
Pb Median (mg/kg)	6.85	7.5	0	0.28	1.4	0.6	0	0
Pb Range (mg/kg)	0 to 19	0 to 24	0 to 1.8	0 to 7.5	0 to 6.5	0 to 5.2	N/A	N/A
ANOVA	$F_{(1, 102)} = 0.324$ $P = 0.571$		$F_{(1, 115)} = 2.278$ $P = 0.134$		$F_{(1, 68)} = 3.009$ $P = 0.0873$		$F_{(1, 7)} = \text{N/A}$ $P = \text{N/A}$	

FIGURES

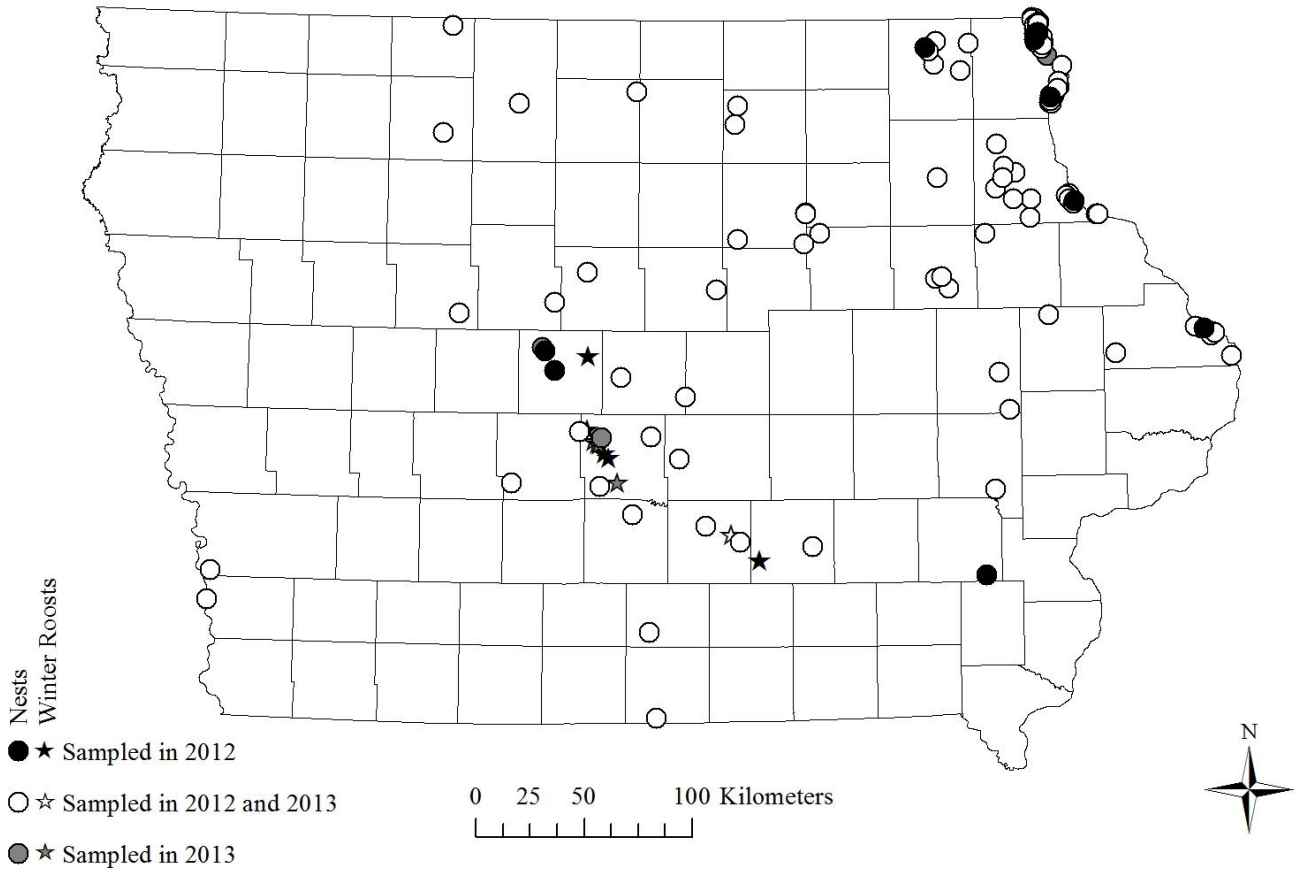

Figure 1. Map of Iowa showing the location of 110 Bald Eagle nests (circles) below which feces were collected in 2012 (n=110) and 2013 (n=107) and the location of 10 Bald Eagles winter roosts (stars) below which feces were collected in 2012 (n=83) and 2013 (n=86). Black circles/stars represent sites only sampled in 2012, white circles/stars represent sites sampled in both 2012 and 2013, and gray circles/stars represent sites only sampled in 2013. Nests were chosen randomly from a list of over 200 nests considered active in 2011 by the U.S. Fish and Wildlife Service and the Iowa Department of Natural Resources. Nests found to be inactive, destroyed, or inaccessible in 2012 and 2013 were replaced with randomly selected backup nests. Winter roosts were chosen opportunistically.

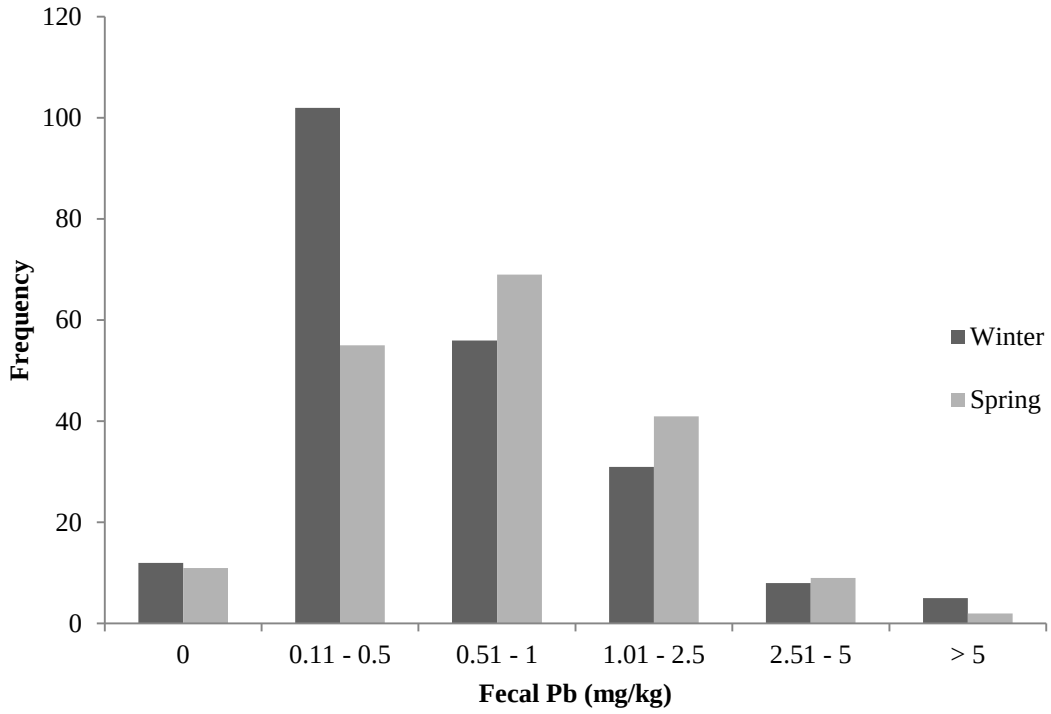

Figure 2. Histogram of lead levels in fecal samples collected from below Iowa Bald Eagle nests sampled in winter (n=214) and spring (n=187) in 2012 and 2013. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (0 mg/kg [(lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards)], 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5 mg/kg, 2.51 - 5 mg/kg, and > 5 mg/kg).

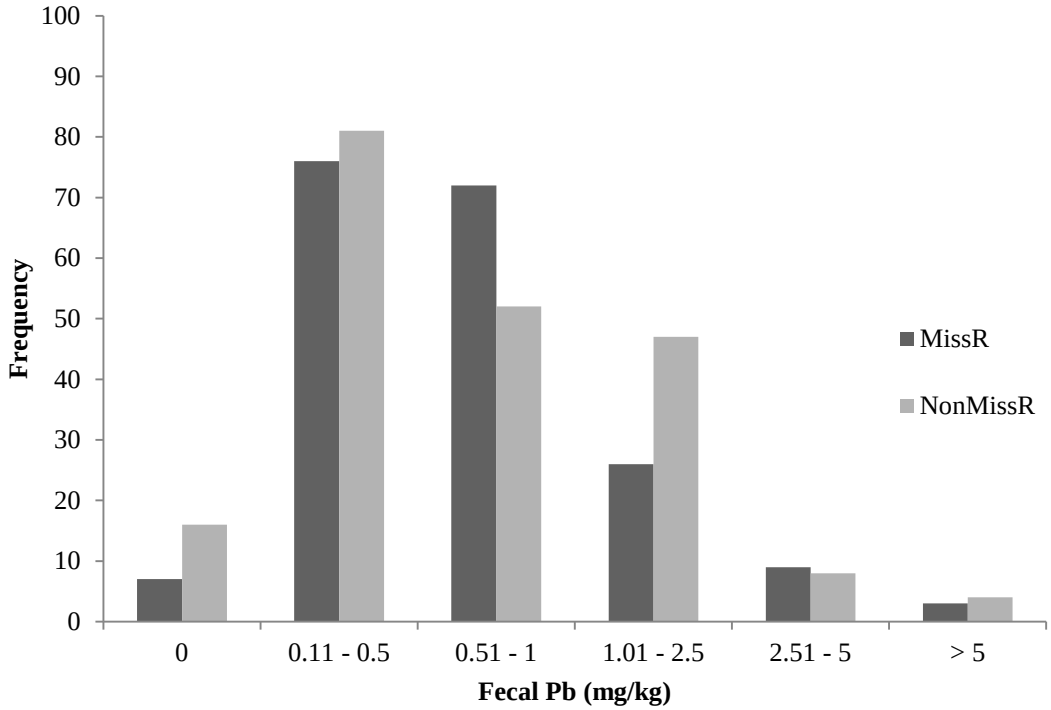

Figure 3. Histogram of lead levels in fecal samples collected from below nesting Bald Eagles adjacent to the Mississippi River (n=193) and non-adjacent to the Mississippi River (n=208) in Iowa in 2012 and 2013. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (0 mg/kg [(lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards)], 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5 mg/kg, 2.51 - 5 mg/kg, and > 5 mg/kg).

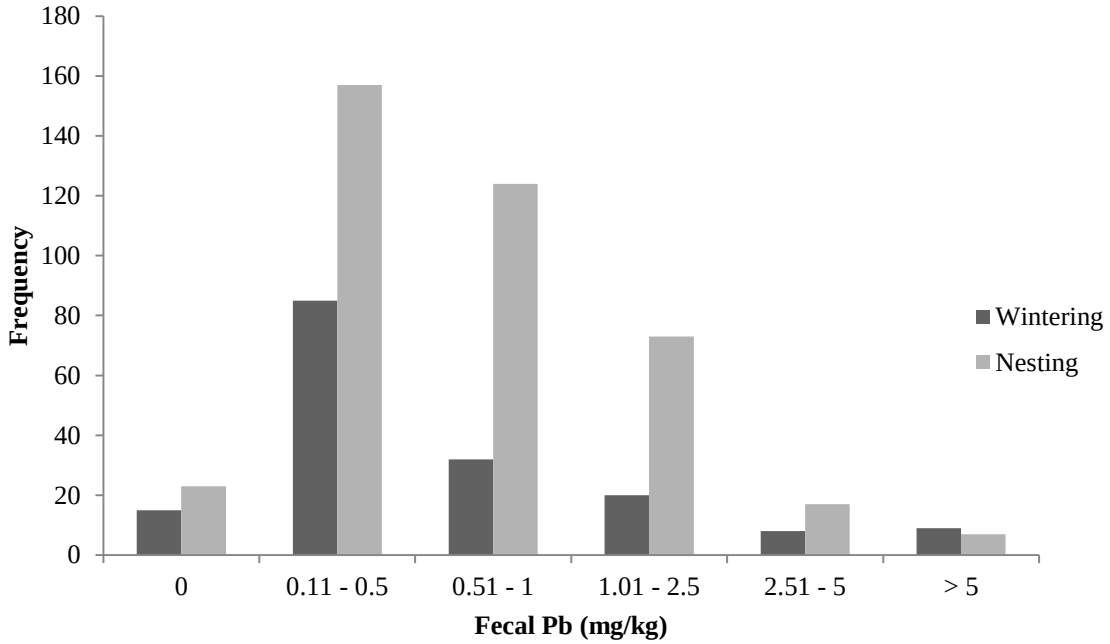

Figure 4. Histogram of lead levels in fecal samples collected from below roosts of wintering (n=169) and nesting (n=401) Bald Eagles in Iowa in 2012 and 2013. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (0 mg/kg [(lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards)], 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5 mg/kg, 2.51 - 5 mg/kg, and > 5 mg/kg).

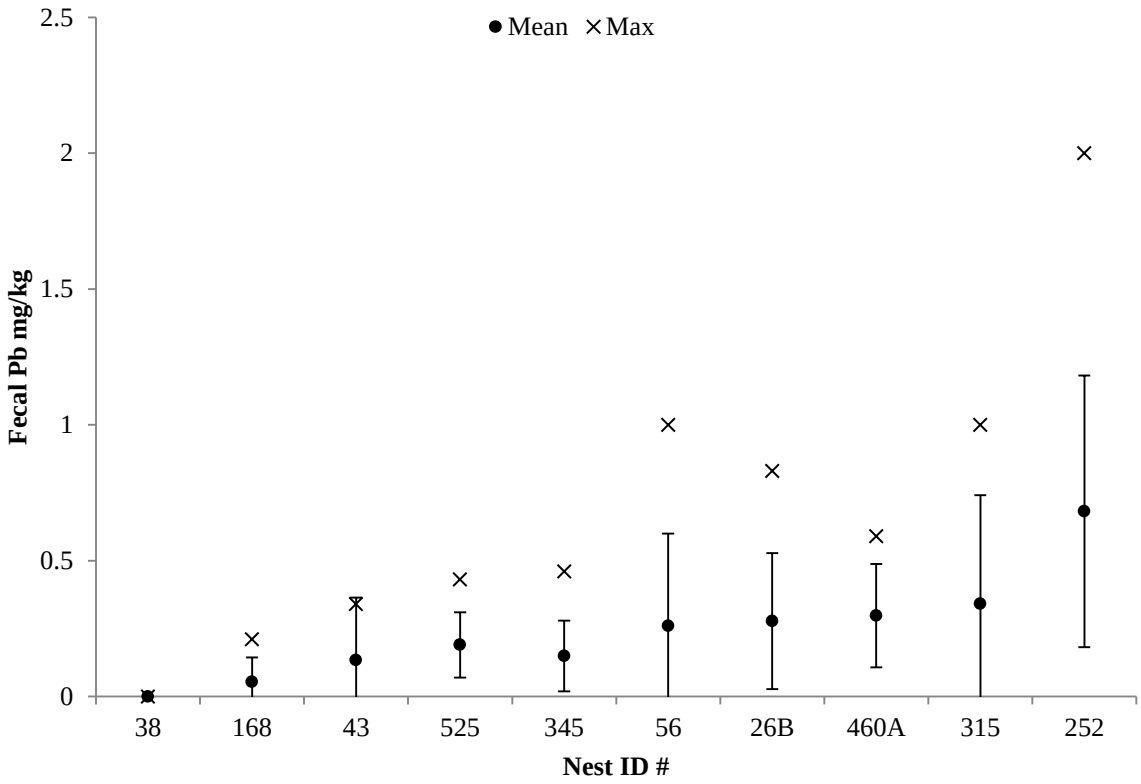

Figure 5. Plot of mean winter fecal lead concentration in samples collected below 10 nests where individual samples (n=70) were opportunistically collected during winter 2013 in Iowa. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Vertical bars represent 95% confidence limits on mean values. Number of samples per nest is as follows: 38 (n=5), 168 (n=8), 43 (n=5), 525 (n=6), 345 (n=6), 56 (n=8), 26B (n=8), 460A (n=8), 315 (n=8), 252 (n=8).

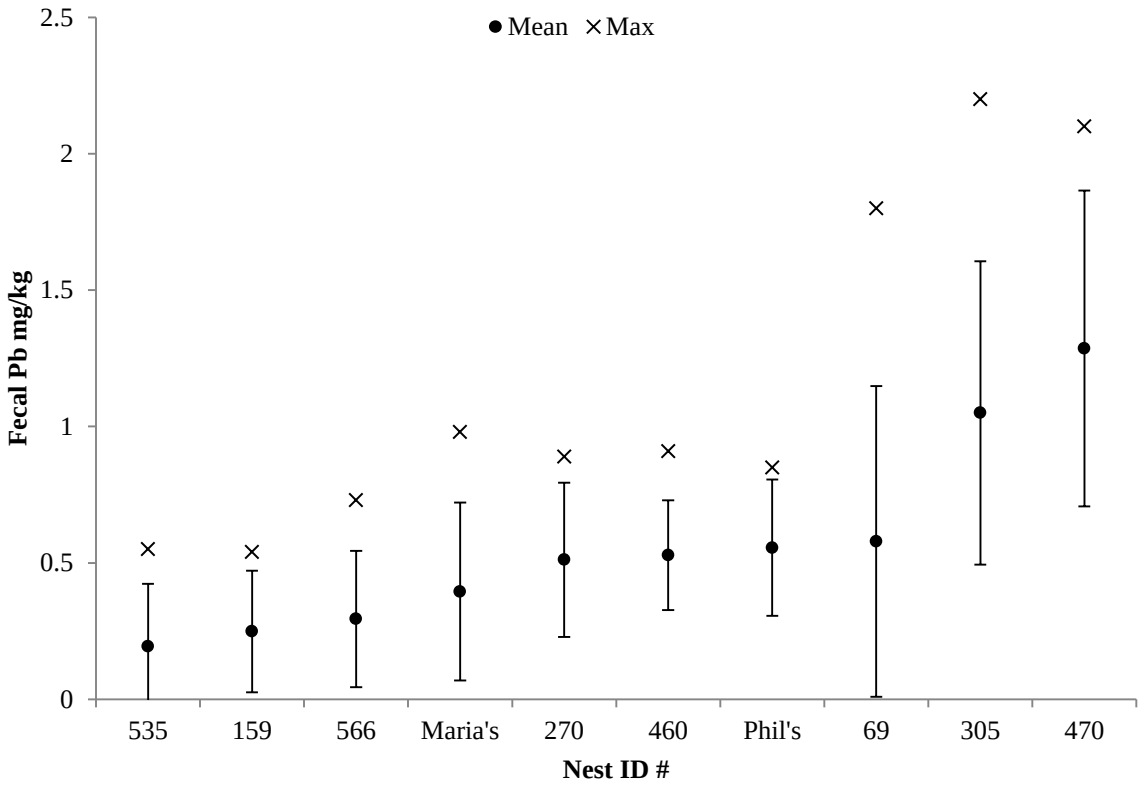

Figure 6. Plot of mean spring fecal lead concentration in samples collected below 10 nests where individual samples (n=79) were opportunistically collected during spring 2013 in Iowa. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Vertical bars represent 95% confidence limits on mean values. Number of samples per nest is as follows: 535 (n=7), 159 (n=7), 566 (n=7), Maria's (n=6), 270 (n=7), 460 (n=7), Phil's (n=7), 69 (n=7), 305 (n=7), 470 (n=7).

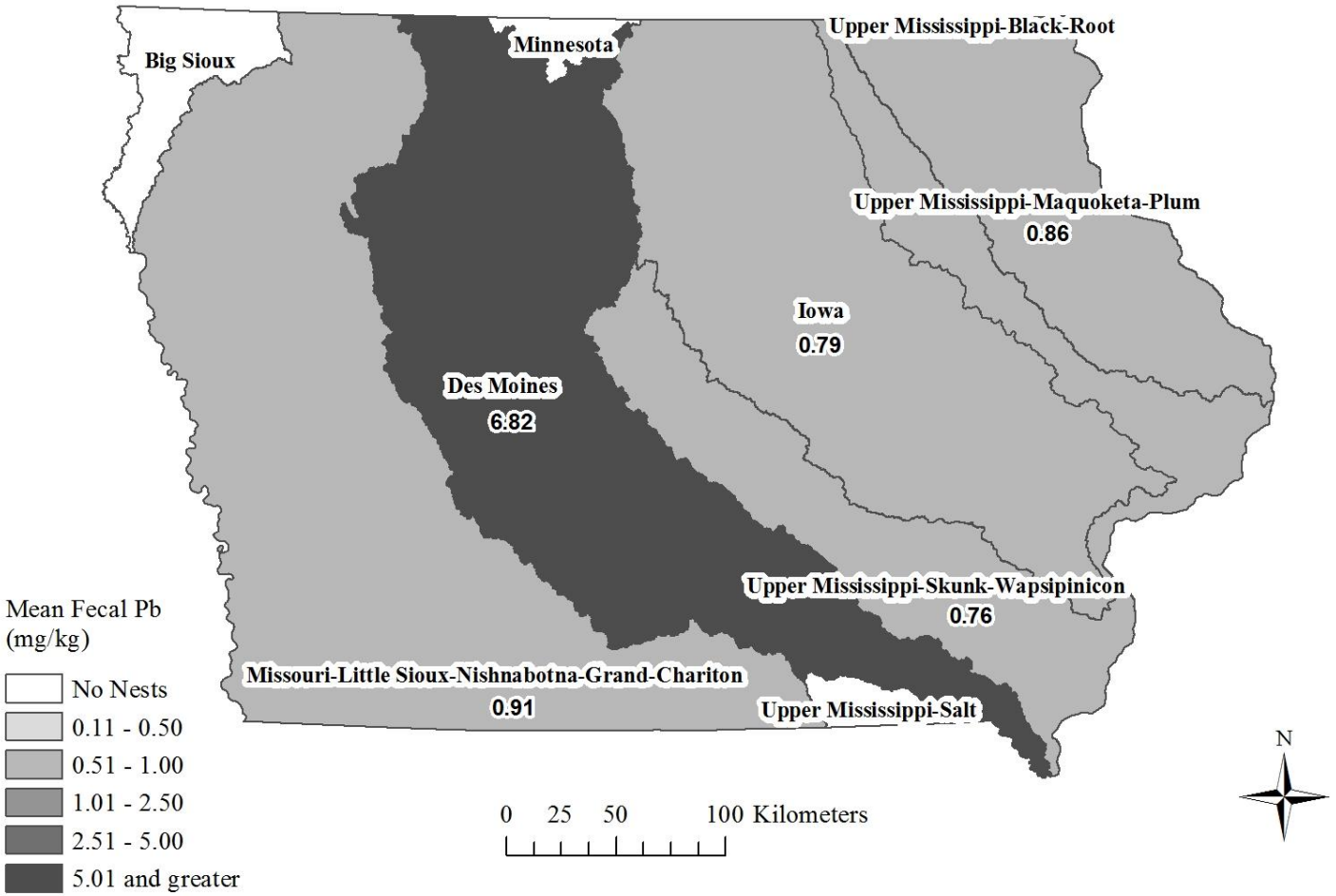

Figure 7. Map of Iowa showing mean winter fecal lead concentration by watershed. Samples were collected from below Bald Eagle nests (n=214) in winter 2012 and 2013. To statistically compare watersheds at the basin level, four watersheds (Missouri-Little Sioux, Missouri-Nishnabotna, Grand, and Chariton) were combined together because they each included only one nest. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (No Nests, 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5 mg/kg, 2.51 - 5 mg/kg, and > 5 mg/kg). Lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards.

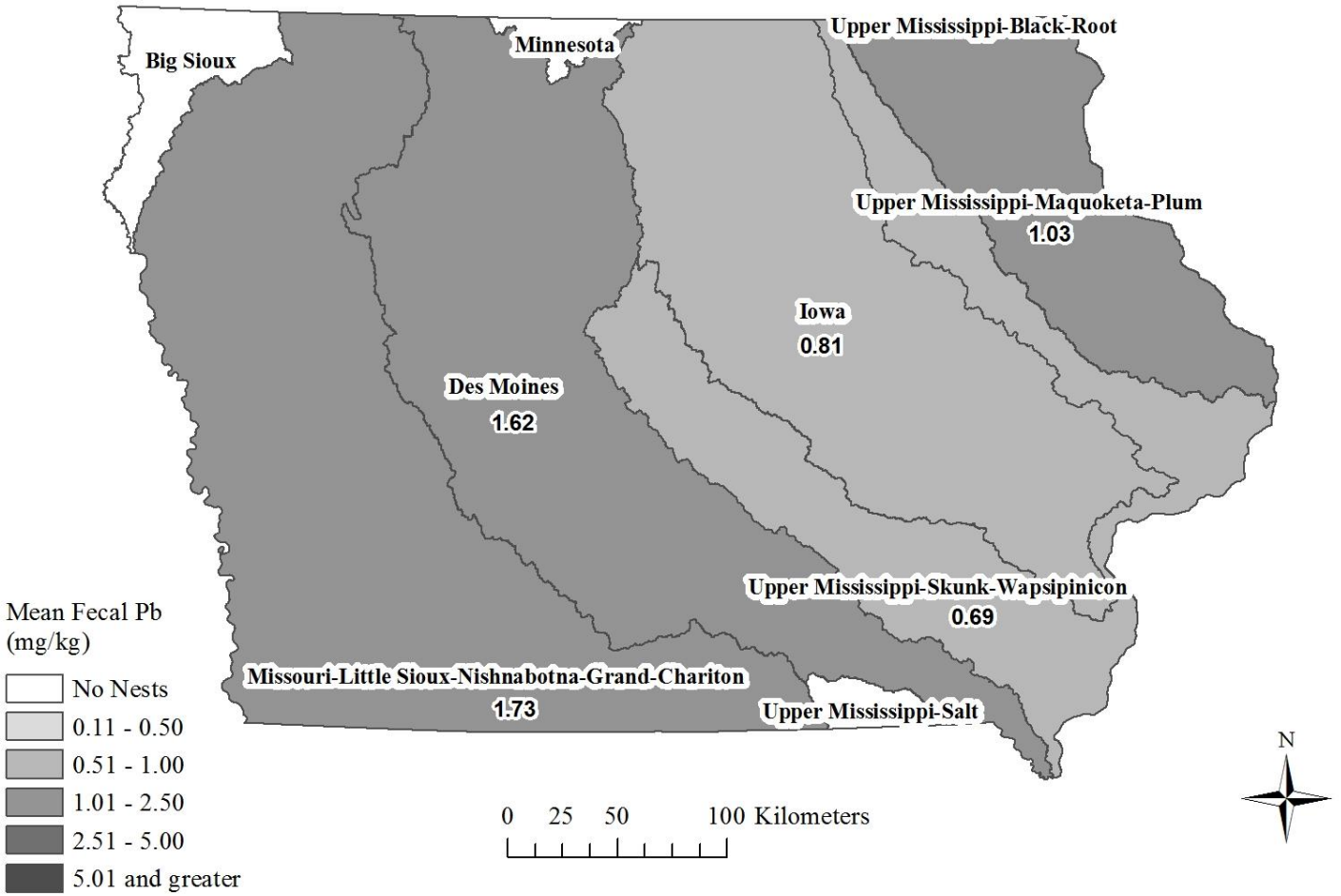

Figure 8. Map of Iowa showing mean spring fecal lead concentration by watershed. Samples were collected from below Bald Eagle nests (n=187) in spring 2012 and 2013. To statistically compare watersheds at the basin level, four watersheds (Missouri-Little Sioux, Missouri-Nishnabotna, Grand, and Chariton) were combined together because they each included only one nest. Fecal lead (Pb) levels are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (No Nests, 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5 mg/kg, 2.51 - 5 mg/kg, and > 5 mg/kg). Lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards.

CHAPTER 3. LEAD EXPOSURE IN FREE-FLYING VERSUS REHABILITATION BALD EAGLES

A paper to be submitted to Ecotoxicology

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ABSTRACT

In North America, raptor rehabilitators continue to report cases of lead exposure in Bald Eagles (*Haliaeetus leucocephalus*), but little is known about levels of lead exposure in free-flying Bald Eagles. To determine if lead levels in Bald Eagles admitted to rehabilitation centers were representative of lead levels in free-flying Bald Eagles, we non-invasively collected fecal samples from the base of Bald Eagle roost and nest trees during 2012 and 2013 and compared their lead levels to the feces of Bald Eagles admitted to three raptor rehabilitation centers in Iowa. We documented lead in 93% of feces from free-flying Bald Eagles and in 100% of the feces collected from rehabilitation Bald Eagles. Fecal lead levels in rehabilitation Bald Eagles were significantly higher than lead levels in free-flying Bald Eagles (20.36 mg/kg vs. 1.34 mg/kg, $P < 0.001$). We also compared blood lead levels with fecal lead levels taken from rehabilitation Bald Eagles and found that fecal lead levels were higher than blood lead levels (21.55 mg/kg vs. 2.87 mg/kg), but these differences were not statistically significant ($P > 0.05$). We created a linear regression examining the relationship between fecal and blood lead levels from rehabilitation Bald Eagles and found that fecal lead levels were a significant predictor of blood lead levels in rehabilitation Bald Eagles. From this regression we created a model to estimate unknown blood lead levels from known fecal

lead levels. Our results suggest that the proportion of the free-flying population exposed to high levels of lead is low and this small subset of eagles is likely the one that gets sent to rehabilitation centers. In addition, our results suggest that feces are a promising method for non-invasively measuring lead exposure in Bald Eagles.

INTRODUCTION

Raptor rehabilitators throughout the U.S. continue to report cases of lead exposure in Bald Eagles and other raptors and have also noticed temporal correlations between the incidence of lead exposure and the timing of upland and big game hunting seasons (Kramer and Redig 1997, Harris and Sleeman 2007, Neumann 2009, Redig et al. 2009, Strom et al. 2009, Stauber et al. 2010). In Iowa, between January 2004 and December 2013, 59% of 278 Bald Eagles admitted to rehabilitation facilities had detectable levels of lead in their blood (Neumann, personal communication). Environmentalists have inferred that lead exposure in Bald Eagles and other raptors may be attributable to ingested lead ammunition (Craig et al. 1990, Clark and Scheuhammer 2003, Harradine et al. 2004, Fisher et al. 2006, Hunt et al. 2006, Saito 2009, Kelly et al. 2011, Nam et al. 2011, Bedrosian et al. 2012, Finkelstein et al 2012, Pagel et al. 2012). While there certainly is cause for concern about the annual influx of lead exposed Bald Eagles to rehabilitation centers, little is known about the level of lead exposure in free-flying Bald Eagles. Because of this lack of information, our understanding of the magnitude of lead exposure in free-flying Bald Eagles may be biased by the subset of birds admitted to wildlife rehabilitators simply because they were found injured, incapacitated, or dead.

Ingesting spent lead ammunition, lead fragments embedded in food items, and lead fishing tackle can all cause morbidity and mortality in birds (Locke 1982, Franson 1996,

Kendall et al. 1996, Vyas 2000, Sanborn 2002, Clark and Scheuhammer 2003, Fisher et al. 2006, Knopper 2006, Cade 2007, Craighead and Bedrosian 2008, Martin et al. 2008, Domenech and Langner 2009, Bedrosian et al. 2012). Bald Eagles can be exposed to lead either through direct ingestion or through indirect ingestion from the tissues of lead exposed prey (Harradine et al. 2004). While most ingested lead is excreted through regurgitation, defecation, or sequestration in feathers, some lead can be retained and accumulates in tissue (Platt 1976, Pattee et al. 1981, Leonzio and Massi 1989, Burger 1993, Pain et al. 1997, Sanderson 2002, Dauwe et al. 2003, Ek et al. 2004, Nam et al. 2004, Mateo et al. 2006, ATSDR 2007, Bergdahl and Skerfving 2008, Martinez-Haro et al. 2010, Route et al. 2011). Unlike waterfowl, raptors do not have a muscular gizzard that can erode lead fragments through the grinding action of grit; their stomach pH is low (pH 1-4) and makes lead very soluble (Clemens et al. 1975, Dieter and Finley 1978, Roscoe et al. 1979, Pain et al. 1997). When lead is not excreted, it is absorbed into the blood stream through the gastrointestinal tract. Dissolved lead is then deposited in soft tissues, such as the liver and kidneys, and ultimately accumulates in the skeleton, especially in areas of active bone formation (Scheuhammer 1987, Eisler 1988, Ek et al. 2004, Martin et al. 2008, Martinez-Haro et al. 2010). Absorption and retention can vary greatly among individuals depending on their age, sex, and diet (Pattee et al. 1981, Eisler 1988, Wayland et al. 1999).

One of the challenges to characterizing the magnitude of lead exposure in free-flying Bald Eagles is obtaining samples. While blood samples might be the most commonly-used biomarker to quantify lead exposure (Bergdahl and Skerfving 2008), the potential for negative impacts resulting from handling birds and climbing nest trees make blood sampling less than ideal when dealing with large birds in a large-scale field study (Richardson and

Miller 1997, Rosenfield et al. 2007). An alternative to blood sampling is to test fecal matter for lead because, similar to blood, it represents acute short-term dietary exposure and when dealing with raptors it can be easier to collect and is non-invasive because it can be collected from the ground below nest and roost trees. To the best of our knowledge, fecal samples have not yet been used as a biomarker for lead exposure in Bald Eagles. But fecal lead levels have been successfully used to measure lead exposure in other species including Blue and Great tits, Great Blue Heron, Greylag Geese, Peregrine Falcon, Pied Flycatcher, Little Blue Heron, and Mallard (Fitzner et al. 1995, Eeva and Lehikoinen 1996, Spahn and Sherry 1999, Dauwe 2000, Sanderson 2002, Ek et al. 2004, Tiller et al. 2005, Mateo et al. 2006, Berglund et al. 2012, Martinez-Haro et al. 2010). Fecal lead levels have also been positively correlated with lead levels in the blood and liver in Greylag Geese and in humans (Mateo et al. 2006, Bergdahl and Skerfving 2008). Given the success of other studies in using feces as a biomarker, we chose to collect fecal matter from free-flying Bald Eagles for this study.

We partnered with professional wildlife rehabilitators, who simultaneously collected blood and fecal samples from Bald Eagles as they were admitted to one of three wildlife rehabilitation centers in Iowa. The samples they collected allowed us to determine how representative rehabilitation Bald Eagles are of free-flying Bald Eagles in terms of lead exposure. We were also able to determine whether feces, as an alternative to blood, could provide comparable information about lead exposure and the presumptive clinical effects of different levels.

METHODS

Study Species

The Bald Eagle is a year-round resident in Iowa, although their numbers vary seasonally. There are an estimated 200-300 active Bald Eagle nests in the state (Shepherd and Nixon 2011, Shepherd 2013) and the number of wintering eagles is in the thousands. Bald Eagle numbers peak in Iowa between November and January, when migrants arrive from their breeding grounds in more northern states and Canada (Jackson et al. 1996). Bald Eagles nest in Iowa from late January through July, with incubation starting in late February. Eagles occur in the greatest concentrations wherever there is open water and an abundant food source (Kent and Dinsmore 1996, Dyar 2010). Bald Eagle nests are most heavily concentrated in northeastern part of the state. Bald Eagles also nest along river corridors throughout the remainder of Iowa, but at lower densities, with the fewest nests occurring in western Iowa (Iowa DNR 2011, 2012).

Fecal sampling of free-flying Bald Eagles

Lead exposure in free-flying Bald Eagles was quantified using fecal samples. All fecal samples were collected from below wintering and nesting Bald Eagles using non-invasive collection techniques to reduce sampling-associated disturbance (Rosenfield et al. 2007). We conducted nest sampling from 16 February to 29 March 2012 (winter) and from 4 May to 22 May 2012 (spring). In 2013, we conducted nest sampling from 20 February to 28 March (winter) and from 1 May to 3 June (spring). For nesting eagles, the first fecal sampling visits occurred during the incubation period and the second sampling visits occurred when the Bald Eagle chicks were 3-9 weeks old. Nest sites were accessed on foot where possible and by boat where necessary at Mississippi River islands and sloughs. We

spent no more than 30 minutes within 100 m of each nest site. Fecal samples below a nest could be from 2-5 individuals depending on the time of the visit. During winter visits 2 adults were present at the nest incubating eggs, whereas during spring 2 adults were present along with 1-3 nestlings. Because of this feces collected below each nest were mixed together into one sample or results from multiple samples were averaged (at 20 nests in 2013), with the resulting lead level being representative of the nest. Feces deposited by wintering Bald Eagles were also opportunistically collected primarily along the Des Moines River from January to March 2012 (6 sites) and from January to March 2013 (5 sites). Each fecal sample collected at winter roosts was considered independent and representative of one bird.

Environmental substrate sampling at Bald Eagle wintering roosts and nests

To better understand background environmental lead levels at sampling sites, we also collected substrate samples (soil, leaves, bark, snow, water) at nesting and wintering locations, specifically focusing on substrates from which we collected feces. For example, if we collected feces from soil and leaves at a site, we also collected soil and leaf samples from the site. This allowed us to compare fecal lead levels from free-flying Bald Eagles to lead levels in the environment.

Blood and fecal sampling of rehabilitation Bald Eagles

In 2012 and 2013, professional wildlife rehabilitators were recruited to opportunistically collect blood and fecal samples from Bald Eagles as they were admitted to one of three raptor rehabilitation centers in Iowa: Saving Our Avian Resources (S.O.A.R.) in Dedham, the Macbride Raptor Project in Cedar Rapids, and the Wildlife Care Clinic in Ames. Feces and blood were collected from eagles regardless of the reason for admittance and prior to any chelation therapy. Because chelation therapy is used to detoxify lead in the

body, it was essential to collect samples from birds submitted for rehabilitation prior to chelation in order to get a baseline lead measurement. In two cases, additional blood and fecal samples from two individual eagles were tested post chelation in order to increase the sample size of paired samples. Up to 1 cc. of blood was collected from each Bald Eagle and fecal samples were collected opportunistically as the eagles defecated on either wax paper or wood chip bedding within their holding pens. All Bald Eagles admitted to rehabilitation centers were found in the wild, incapacitated by injury, starvation, or mobility issues related to toxicant exposure (lead and organophosphates [S.O.A.R., Macbride Raptor Project, and Wildlife Care Clinic, personal communication]). We hypothesize that lead levels will be positively correlated with blood lead levels though this relationship may not be 1:1.

Sample preparation and lead testing

All fecal samples from free-flying and rehabilitation eagles were inspected under a dissecting microscope and cleaned of any substrate that did not pass through the bird (i.e., leaves, bark, twigs, and soil) to minimize background environmental contamination that might bias results. Processed feces, blood, and environmental substrate samples were transferred to the State Hygienic Laboratory (SHL) at the University of Iowa where lead levels were determined using inductively coupled plasma mass spectrometry (ICP-MS). The testing process involved an acid digestion using diluted nitric acid to break down any solid matter in the sample and allow any lead particles to go into solution. The solution was ionized by cooling it to 2 °C and then pumping it into plasma where it was heated to 5,000-7,000 °C. Any lead ions in the sample were counted by the mass spectrometer. For solids (feces and substrates), the SHL typically reports lead levels below 0.94 mg/kg as <1.0 mg/kg, because that threshold represents their reporting limit, or ability to discern the difference

between background noise and a valid signal attributable to the analyte of interest. Lead levels from 0.95 - 1 mg/kg are reported as 1 mg/kg, and lead levels >1.0 mg/kg are reported as actual values. For statistical analysis, we used the instrument read-out values for all samples measuring <1.0 mg/kg acknowledging that there might be some error at these small concentrations. Lead levels from 0.0 to 0.1 mg/kg were considered effectively zero based on the SHL testing standards. Blood lead levels were reported in µg/dl and were converted to ppm and then mg/kg for comparison with fecal lead levels (i.e: Reported blood lead = 28 µg/dl = 28/100 = 0.28 ppm = 0.28 mg/kg).

Data Analyses

Lead levels were compared a) between fecal samples collected from free-flying Bald Eagles and rehabilitation Bald Eagles, and b) between fecal samples and blood samples from rehabilitation Bald Eagles using ANOVAs ($\alpha = 0.05$) in R-Studio (RStudio 2013). We also calculated means, 95% confidence limits, medians, and ranges for each group. We then used a linear regression to estimate the relationship between paired blood and fecal lead levels and created a model for estimating unknown blood lead levels from known fecal lead levels.

It was not economically feasible to test all environmental substrates collected, so we tested 1-2 substrates from any nest site with fecal lead levels >1.0 mg/kg and one substrate from a random selection of nest sites that had fecal lead levels <1.0 mg/kg. We calculated means, 95% confidence limits, medians, and ranges for each substrate type sampled (soil, leaves, bark, snow), and compared lead levels (by substrate type) from free-flying sites that had fecal lead levels of >1.0 mg/kg versus <1.0 mg/kg using an ANOVA.

The sampling procedures described above were conducted with permission from the U.S. Fish and Wildlife Service (permit #MB52842A-0) and the State of Iowa (permit #SC-871 and #SC-872).

RESULTS

We tested 570 samples from free-flying Bald Eagles (nest samples = 401 [from 107 nests in 2012 and 107 nests in 2013], wintering = 169) for lead in 2012 and 2013. We also tested 51 fecal samples and 47 blood samples from 48 rehabilitation Bald Eagles for lead in 2012 and 2013. We documented lead (>0.11 mg/kg) in 93% of the fecal samples collected from free-flying Bald Eagles and in 100% of the fecal samples collected from rehabilitation Bald Eagles (Figure 1). Lead levels from wintering and nesting Bald Eagles were not statistically different from each other ($P > 0.05$) or between years ($P > 0.05$). We combined wintering and nesting samples for the analyses. Fecal lead levels from rehabilitation Bald Eagles were also not statistically different between years ($P > 0.05$), so both years were combined for analyses. We summarized lead levels in free-flying and rehabilitation bird samples using relative frequencies for each group and arbitrarily binned samples into six groups (0 mg/kg [0.0 up to 0.1], 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01 - 2.5 mg/kg, 2.51 - 5 mg/kg, and >5 mg/kg; Figure 1).

Fecal lead levels in rehabilitation Bald Eagles were significantly higher than fecal lead levels in free-flying Bald Eagles (mean = 20.36 mg/kg vs. 1.34 mg/kg, $P < 0.001$; Table 1). The five highest fecal lead levels among rehabilitation samples were 520 mg/kg, followed by 140, 106, 74, and 40 mg/kg, compared to the five highest fecal lead levels among free-flying samples, which were 170 mg/kg, followed by 25, 22, 19 and 15 mg/kg. We found that 79% of blood samples from rehabilitation Bald Eagles had lead levels <1.0 mg/kg, while

21% had lead levels >1.0 mg/kg. Blood lead levels from rehabilitation eagles were not statistically different between years ($P > 0.05$), so both years were combined for analysis. Fecal lead levels were higher than blood lead levels in rehabilitation Bald Eagles (mean = 20.36 mg/kg vs. 2.87 mg/kg) but these differences were not statistically significant ($P > 0.05$; Table 2).

To account for unequal variance between blood lead levels and fecal lead levels and to correct for skewness in the fecal data, all lead levels from rehabilitation Bald Eagles were $\log_{10}$ transformed. The resulting regression showed that fecal lead levels were a significant predictor of blood lead levels in rehabilitation Bald Eagles ($\beta = 0.71$, $SE = 0.068$, $P < 0.001$, $R^2 = 0.71$; Figure 2). From this regression of rehabilitation blood samples versus fecal samples we created the model,

$$log_{10}Blood = (0.71 * log_{10}Feces) - 0.48$$

Using this model, we estimated $\log_{10}$ blood levels for a range of $\log_{10}$ fecal levels equivalent to the range found in our free-flying fecal samples (0-170 mg/kg) and then back-transformed them to actual blood lead values (in mg/kg). We also calculated $\log_{10}$ transformed 95% confidence limits for each blood lead estimate using the standard error for the slope of the original regression equation. The resulting $\log_{10}$ transformed 95% confidence limits were then back transformed. Using this model, estimates of free-flying blood lead levels were calculated from the fecal lead levels found in free-flying eagles (n=570) in 2012 and 2013 (Figure 3). As fecal lead levels increased the variance around the estimated blood lead level estimates also increased.

In 2012 and 2013, we tested 1-2 environmental substrates (n=171), from nest sites that had fecal lead levels of >1.0 mg/kg and one substrate sample was also tested for 113

randomly selected nest sites that had fecal lead levels of <1.0 mg/kg. We also tested 16 substrates from wintering eagle sites during 2012 and 2013. We documented lead in 79% of environmental substrates collected from below nests and winter roosts in Iowa in 2012 and 2013 (Table 3). Soil samples had the highest mean lead level (7.05 mg/kg, 95% CL [6.23, 7.87]), followed by bark (1.55 mg/kg, 95% CL [1.16, 1.95]), leaves (0.56 mg/kg, 95% CL [0.36, 0.75]), and snow (0 mg/kg, 95% CL [0, 0]). Soil lead levels were higher at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels <1.0 mg/kg (mean = 7.41 mg/kg vs. 6.97 mg/kg), but these differences were not statistically significant ($P > 0.05$). Leaf lead levels were also higher at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels <1.0 mg/kg (mean = 0.61 mg/kg vs. 0.31 mg/kg), but these differences were also not statistically significant ($P > 0.05$). We found bark lead levels to be lower at nest sites and winter roosts with fecal lead levels >1.0 mg/kg compared to those with fecal lead levels <1.0 mg/kg (mean = 1.28 mg/kg vs. 1.96 mg/kg), but these differences were not statistically significant ($P > 0.05$). All snow samples had lead levels <0.1 mg/kg.

DISCUSSION

We found various lead levels in the feces of free-flying and rehabilitation Bald Eagles in Iowa. Fecal lead levels in rehabilitation Bald Eagles were significantly higher than fecal lead levels in free-flying Bald Eagles. Compared to rehabilitation Bald Eagles, the majority of free-flying Bald Eagles had low fecal lead levels with the exception of a few individuals. This suggests that lead levels in rehabilitation Bald Eagles are not representative of lead exposure levels in free-flying Bald Eagles, but rather representative of a small subset of the population. This small subset of eagles was admitted to rehabilitation centers because they

were found debilitated in the wild, either by injury, starvation, or toxicant exposure (S.O.A.R., Macbride Raptor Project, and Wildlife Care Clinic, personal communication). Furthermore, the fecal lead levels we documented in most free-flying eagles were low and within the range of fecal lead levels documented in other birds inhabiting non-lead contaminated sites (Fitzner et al. 1995, Dauwe et al. 2000, Ek et al. 2004, Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010). Free-flying fecal lead levels were also similar to the environmental lead levels we documented in substrates collected from below nests and winter roosts.

We also found various lead levels in the blood of rehabilitation Bald Eagles. In 1997, Kramer and Redig retroactively reviewed 138 cases of lead-exposed Bald and Golden eagles admitted to The Raptor Center at the University of Minnesota to identify the relationships between blood lead levels and clinical symptoms. They reported that during 16 years, blood lead levels >1.2 mg/kg were always fatal, while levels between 0.61 and 1.2 mg/kg represented clinical and treatable lead poisoning, and levels between 0.2 and 0.6 mg/kg represented subclinical lead exposure. In Iowa, S.O.A.R considers any blood lead level >1.0 mg/kg to be abnormal and starts chelation treatment for any eagle with blood lead levels of 0.2 mg/kg or greater (Neumann 2009). In the blood of rehabilitation Bald Eagles that we tested, we found 21% had blood lead levels >1.2 mg/kg (1 was released, 7 died, and 2 were euthanized), 13% had blood lead levels between 0.77 - 0.61 mg/kg (2 were released, 1 died, and 3 were euthanized), 36% had blood lead levels between 0.6 - 0.2 mg/kg (8 were released, 1 was permanently crippled and became an educational bird, 3 died, and 5 were euthanized) , and 30% had blood lead levels <0.2 mg/kg (4 were released, 1 was permanently crippled and became an educational bird, 2 died, and 7 were euthanized). While we found that the mean

blood lead level was lower than the mean fecal lead level in the rehabilitation Bald Eagles, due to the high variation in the fecal samples, these groups were not statistically different.

In human adults, blood lead levels at 40 μg/dL (0.4 mg/kg) are known to cause motor nerve dysfunction, while blood lead levels ≥40 μg/dL have been associated with increased risk for cancer, cardiovascular, and all-cause mortality (CDC 2012). In children, there is currently no safe blood lead threshold and strong negative correlations between blood lead levels and test scores for math, reading, and comprehension have been noted at levels as low as 2.5 μg/dL dL (0.025 mg/kg)(Patrick 2006). Adverse effects have also been documented in pregnant and lactating women at levels <10 μg/dL (<0.1 mg/kg) (CDC 2012).

The regression model we created from fecal and blood lead levels found in rehabilitation Bald Eagles can be useful in estimating blood lead levels from the fecal lead levels found in free-flying Bald Eagles, but only with caution. The fecal lead mean for free-flying Bald Eagles was 1.34 mg/kg, which would correspond to a blood lead estimate of 0.41 mg/kg (95% CL ([0.30, 0.56]) in our model. That blood lead estimate and its 95% confidence limits correspond with the subclinical lead exposure group as described by Kramer and Redig (1997). However, using the mean may be inappropriate because the lead levels in most free-flying eagles were low and the mean was biased by a few high cases, one being extreme. A more conservative approach would be to estimate blood lead levels using the median, which was 0.52 mg/kg in the feces of free-flying Bald Eagles. That median would correspond to a blood lead estimate of 0.21 mg/kg (95% CL ([0.15, 0.29]), which is very near the lower threshold of the subclinical lead exposure group as described by Kramer and Redig (1997). We feel that using the median is more appropriate in this case and a more realistic estimate of typical blood lead levels in free-flying Bald Eagles in Iowa. However, the means estimated

by the model have considerable variation associated with them, especially at higher lead levels. As a result, there is not likely to be a perfect correlation between a given fecal lead level and a blood level. To a degree, more samples will help improve the model by lowering the variation, but individual variation and time since lead exposure relative to when sampling takes place all likely contribute to noise in the estimate. The model does indicate that when fecal lead levels are very low, it is likely that blood lead levels will also be very low. When fecal lead levels are very high, it is likely that blood lead levels will also be high, but there is greater variation in blood lead estimates at higher levels than at low levels.

In other avian studies where lead exposure was quantified, fecal lead levels <5 mg/kg generally occurred when the birds inhabited study sites considered to be reference (i.e., non-lead contaminated) sites (Fitzner et al. 1995, Dauwe et al. 2000, Ek et al. 2004, Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010). Overall, we found 16 (2.8%) nesting and wintering fecal samples with lead levels greater than 5.0 mg/kg (9 between 5.1-10.0 mg/kg, 4 between 10.1-20.0 mg/kg, 2 between 20.1-25.0 mg/kg, and one measuring 170.0 mg/kg) out of 570 total samples collected from nesting and wintering Bald Eagles. While we do not know the fate of any wintering Bald Eagles sampled, we did record the short-term fate of most nesting Bald Eagles sampled. For example, the sample with the highest fecal lead level (170 mg/kg) was collected from a nest during the winter of 2012. When that nest was revisted in the spring, the fecal lead level was 0.81 mg/kg and the nest was still active with one adult and at least one nestling present. That same nest was active again in winter 2013 with another nestling observed in the spring. The next two highest nesting fecal lead levels (22 mg/kg and 14 mg/kg) were both from samples collected during the spring, when both nests were active with 2 and 1 nestlings, respectively. The next two highest nesting fecal lead

levels (11 mg/kg and 8 mg/kg) were both collected during the winter and during our spring visit we found both nests to still be active and the associated spring fecal samples showed much lower lead levels (0.59 mg/kg and 0 mg/kg, respectively). While we do not know the ultimate fate of the adults or their nestlings, these anecdotes about nesting activity suggest that high fecal lead levels recorded at one time point do not neccesarily indicate chronically high levels of lead or result in nest failure. In total, of the 207 nests we sampled in the winter and revisited in the spring of 2012 and 2013, 83% were still active with nestlings. Similarly, Tiller et al. (2005) reported that despite the proximity of Great Blue Heron colonies to nine retired plutonium production reactors (a past contributor of heavy metals into the environment), these colonies had among the highest reproductive health in the continental U.S., suggesting that low levels of lead (1.0, 1.3, 3.5 mg/kg) in feces did not negatively affect nesting success. It is well established that lead absorption and retention can vary greatly among individuals depending on their age, sex, and diet (Pattee et al. 1981, Eisler 1988, Wayland et al. 1999). It follows that excretion ability varies among individuals too. Our results suggest that the proportion of free-flying Bald Eagles in Iowa exposed to high levels of lead is low and this small subset of eagles is the one that tends to get sent to rehabilitation centers because they are debilitated and unable to fly. The significant relationship between fecal and blood lead levels suggests that feces are a promising approach for non-invasively measuring lead exposure in free-flying Bald Eagles. Feces may also be a valuable tool for testing rehabilitation birds, when blood is difficult to collect, or in large-scale field studies involving free-flying raptors or other large birds in the wild. Our model for estimating blood lead levels, and thus presumptive clinical implications of lead exposure from fecal lead

levels, can be improved with additional paired fecal and blood samples from both rehabilitation and free-flying Bald Eagles.

LITERATURE CITED

Bedrosian, B., D. Craighead, and R. Crandall. 2012. Lead exposure in bald eagles from big game hunting, the continental implications and successful mitigation efforts. PLoS ONE 7(12): e51978. doi:10.1371/journal.pone.0051978.

Bergdahl, I.A., and S. Skerfving. 2008. Biomonitoring of lead exposure-alternatives to blood. Journal of Toxicology and Environmental Health, Part A 71:1235-1243.

Brown, M.J., and S. Margolis. 2012. Lead in drinking water and human blood lead levels in the United States. Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report. <http://www.cdc.gov/mmwr/preview/mmwrhtml/su6104a1.htm>. Accessed 17 April 2014.

Burger, J. 1993. Metals in avian feathers: bioindicators of environmental pollution. Reviews in Environmental Toxicology 5:203-311.

Clark, A.J., and A.M. Scheuhammer. 2003. Lead poisoning in upland-foraging birds of prey in Canada. Ecotoxicology 12:23-30.

Craig, T. H., J. W. Connelly, E. H. Craig, and T. L., Parker. 1990. Lead Concentrations in Golden and Bald Eagles. The Wilson Bulletin 102:130-133.

Dauwe, T., L. Bervoets, R. Blust, R. Pinxten, and M. Eens. 2000. Can excrement and feathers of nestling songbirds be used as biomonitors for heavy metal pollution? Archives of Environmental Contamination and Toxicology 39:541-546.

Dauwe, T., L. Bervoets, R. Pinxten, R. Blust, and M. Eens. 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. Environmental Pollution 124:429-436.

Dyar, J. 2010. Bald Eagles in Iowa. Iowa Department of Natural Resources.

Eeva, T., and E. Lehikoinen. 1996. Growth and mortality of nestling great tits (*Parus major*) and pied flycatchers (*Ficedula hypoleuca*) in a heavy metal pollution gradient. Oecologia 102:312-323.

Ek, K.H., G.M. Morrison, P. Lindberg, and S. Rauch. 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Archives of Environmental Contamination and Toxicology 47:259-269.

Finkelstein, M.E., D.F. Doak, D. George, J. Burnett, J. Brandt, M. Church, J. Grantham, and D.R. Smith. 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. PNAS 109:11449-11454.

Fisher, I.J., D.J. Pain, and V.G. Thomas. 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biological Conservation 131:421-432.

Fitzner, R.E., R.H. Gray, and W.T. Hinds. 1995. Heavy metal concentrations in great blue heron castings in Washington state: a technique for monitoring regional and global trends in environmental contaminants. Bulletin of Environmental Contamination and Toxicology 55:398-403.

Harradine, J. 2004. Spent lead shot and wildlife exposure and risks. Pages 119-130 in Scheinosst, A. (ed.), Proceedings of the World Symposium on Lead Ammunition. World Forum on the Future of Sport Shooting Activities. September 9-10, 2004. Rome, Italy.

Harris, M. C. and J. M. Sleeman. 2007. Morbidity and mortality of Bald Eagles (*Haliaeetus leucocephalus*) and Peregrine Falcons (*Falco peregrinus*) admitted to the wildlife center of Virginia, 1993–2003. Journal of Zoo and Wildlife Medicine 38:62–66.

Hunt, W. G., W. Burnham, C. N. Parish, K. K. Burnham, B. Mutch, and J. L. Oaks. 2006. Bullet fragments in deer remains: implications for lead exposure in avian scavengers. Wildlife Society Bulletin 34:167–170.

Iowa Department of Natural Resources (Iowa DNR). 2011. Unpublished bald eagle nesting data.

Iowa Department of Natural Resources (Iowa DNR). 2012. Unpublished bald eagle nesting data.

Jackson, L.S, C.A. Thompson, J.J. Dinsmore, B.L. Ehresman, J. Fleckenstein, R. Cecil, L.M. Hemesath, S.J. Dinsmore. 1996. The Iowa Breeding Bird Atlas. University of Iowa Press. Iowa City, IA. 484 p.

Kent, T.H. and J.J. Dinsmore. 1996. Birds in Iowa. Self-published. Iowa City and Ames, IA. 391 p.

Kramer, J. L., and P. T. Redig. 1997. Sixteen years of lead poisoning in eagles, 1980–95: an epizootiologic view. Journal of Raptor Research 31:327-332.

Martinez-Haro, M., M.A. Taggart, and R. Mateo. 2010. Pb-Al relationships in waterfowl feces discriminate between sources of Pb exposure. Environmental Pollution 158:2485-2489.

Mateo, R., M.A. Taggart, A.J. Green, C. Cristofol, A. Ramis, H. Lefranc, J. Figuerola, and A.A. Meharg. 2006. Altered porphyrin excretion and histopathology of Greylag geese (*Anser anser*) exposed to soil contaminated with lead and arsenic in the Guadalquivir Marshes, southwestern Spain. Environmental Toxicology and Chemistry 25:203-212.

Nam DH, J. Rutkiewicz, N. Basu. 2011. Multiple metals exposure and neurotoxic risk in bald eagles (*Haliaeetus leucocephalus*) from two Great Lakes states. Environmental Toxicology and Chemistry 31:623-631.

Neumann, K. 2009. Bald Eagle lead poisoning in winter. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0119.

Pagel, J.E., P.B. Sharpe, D.K. Garcelon, A.E. Little, S.K. Taylor, K.R. Faulkner, and C.S. Gorbics. 2012. Exposure of bald eagles to lead on the Northern Channel Islands, California. Journal of Raptor Research 46(2): 168-176.

Patrick, L. 2006. Lead toxicity, a review of the literature. part 1: Exposure, evaluation, and treatment. Alternative Medicine Review 11:2-22.

Redig, P. T., D. R. Smith, and L. Cruz-Martinez. 2009. Potential sources of lead exposure for Bald Eagles: A retrospective study. Extended abstract in R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI 10.4080/ilsa.2009.0208.

Richardson, C. T., and C. K. Miller. 1997. Recommendations for protecting raptors from human disturbance: a review. Wildlife Society Bulletin 25:634-638.

Rosenfield, R. N., J. W. Grier, and R. W. Fyfe. 2007. Reducing management and research disturbance. Pages 351-364 in Raptor Research and Management Techniques. Hancock House Publishers. Surrey, Canada.

RStudio. 2013. RStudio: Integrated development environment for R. Boston, MA. Version 0.97.236, http://www.rstudio.org/

Sanderson, G.C. 2002. Effects of diet and soil on the toxicity of lead in mallards. Ecotoxicology 11:11-17.

Shepherd, S. 2013. Bald eagle (*Haliaeetus leucocephalus*) nesting in Iowa 2013. Iowa Department of Natural Resources. <http://www.iowadnr.gov/Portals/idnr/uploads/wildlife/research/BENestSummary_2012.pdf>. Accessed 28 January 2014.

Spahn S.A. and T.W. Sherry. 1999. Cadmium and lead exposure associated with reduced growth rates, poorer fledgling success of little blue heron chicks (*Egretta caerula*) in South Louisiana Wetlands. Archives of Environmental Contamination and Toxicology 37:377-384.

Stauber, E., N. Finch, P. a. Talcott, and J. M. Gay. 2010. Lead poisoning of bald (*Haliaeetus leucocephalus*) and golden (*Aquila chrysaetos*) eagles in the U.S. Inland Pacific Northwest Region—An 18-year retrospective study: 1991–2008. Journal of Avian Medicine and Surgery 24:279-287.

Strom, S. M., J.A. Langenberg, N.K. Businga, and J.K. Batten. 2009. Lead exposure in Wisconsin birds. In R. T. Watson, M. Fuller, M. Pokras, and W. G. Hunt (Eds.). Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA. DOI10.4080/ilsa.2009.0205.

Temple, D.J., and R.J. Gutierrez. 2004. Factors related to fecal corticosterone levels in California Spotted Owls: implications for assessing chronic stress. Conservation Biology 18:538-547.

TABLES

Table 1. Summary of fecal lead statistics for free-flying and rehabilitation Bald Eagle samples collected in 2012 and 2013. Data from both years were combined together because there were no statistical differences between years. Counts, means, 95% confidence limits, medians, and ranges are presented below with ANOVA results ($\alpha = 0.05$). (Pb = lead, mg/kg = milligram/kilogram)

	Free-flying	Rehabilitation
# of samples	570	51
Pb Mean (mg/kg)	1.34	20.36
Confidence Limits (95%)	0.73, 1.95	0.00, 41.76
Pb Median (mg/kg)	0.52	0.83
Pb Range (mg/kg)	0 to 170	0 to 520
ANOVA	$F_{(1, 619)} = 32.71$ $P < 0.001$	

Table 2. Summary of blood lead and fecal lead statistics from rehabilitation Bald Eagles samples in 2012 and 2013. Data from both years were combined together because there were no statistical differences between years. Counts, means, 95% confidence limits, medians, and ranges are presented below with ANOVA results ($\alpha = 0.05$). (Pb = lead, mg/kg = milligram/kilogram)

	Blood	Feces
# of samples	47	51
Pb Mean (mg/kg)	2.87	20.36
Confidence Limits (95%)	0.64, 5.10	0.00, 41.76
Pb Median (mg/kg)	0.31	0.83
Pb Range (mg/kg)	0 to 37	0 to 520
ANOVA	$F_{(1, 96)} = 2.46$ $P = 0.12$	

Table 3. Summary of substrate lead statistics from nesting and wintering Bald Eagles in Iowa in 2012 and 2013. Count, means, 95% confidence limits, medians, and ranges are presented below along with ANOVA results ($\alpha = 0.05$) comparing lead levels by substrate type between nest and wintering eagle roost sites that had fecal lead levels of >1.0 mg/kg versus <1.0 mg/kg. (Pb = lead, mg/kg = milligram/kilogram)

	Soil <1.0 mg/kg	Soil >1.0 mg/kg	Leaves <1.0 mg/kg	Leaves >1.0 mg/kg	Bark <1.0 mg/kg	Bark >1.0 mg/kg	Snow <1.0 mg/kg	Snow >1.0 mg/kg
# of samples	56	48	34	83	28	42	6	3
Pb Mean (mg/kg)	6.97	7.41	0.31	0.61	1.96	1.28	0	0
CL(95%)	6.12, 7.82	6.07, 8.75	0.13, 0.49	0.37, 0.85	1.28, 2.63	0.8, 1.75	0, 0	0, 0
Pb Median (mg/kg)	6.85	7.5	0	0.28	1.4	0.6	0	0
Pb Range (mg/kg)	0 to 19	0 to 24	0 to 1.8	0 to 7.5	0 to 6.5	0 to 5.2	N/A	N/A
ANOVA	$F_{(1, 102)} = 0.324$, $P = 0.571$		$F_{(1, 115)} = 2.278$, $P = 0.134$		$F_{(1, 68)} = 3.009$, $P = 0.0873$		$F_{(1, 7)} = N/A$, $P = N/A$	

FIGURES

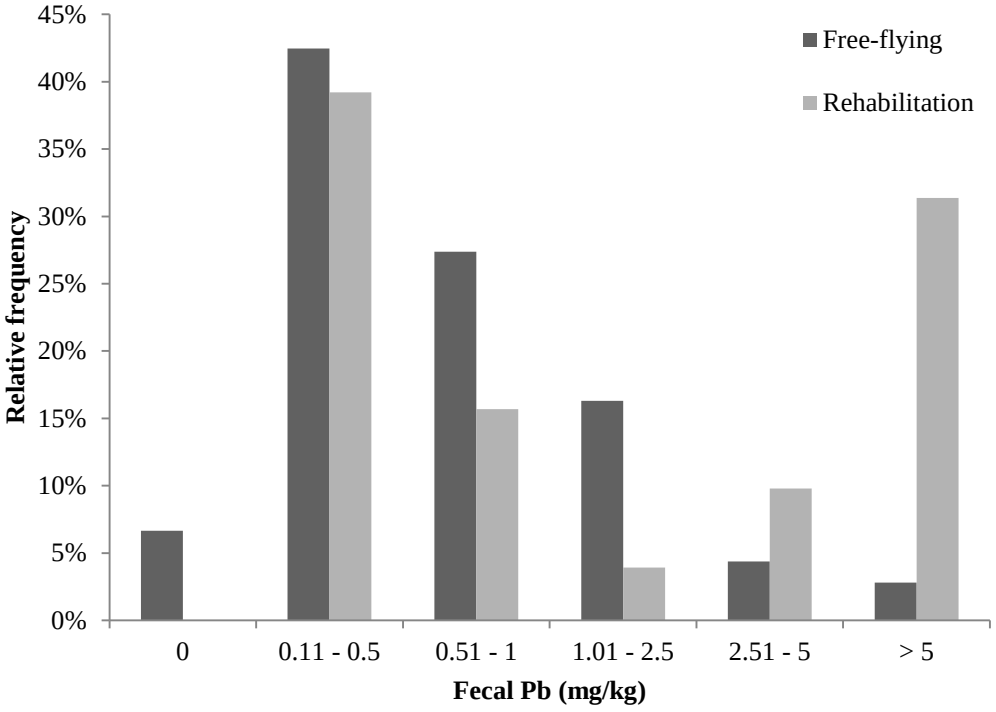

Figure 1. Histogram showing relative frequencies of lead concentrations in fecal samples collected in Iowa from below roosts and nests of free-flying Bald Eagles (n=570) and rehabilitation Bald Eagles (n=51) in 2012 and 2013. Fecal lead (Pb) concentrations are presented in milligram/kilogram (mg/kg). Samples were arbitrarily binned into six groups (0 mg/kg [(lead levels from 0.0 up to 0.1 were considered effectively zero based on the SHL testing standards)], 0.11 - 0.5 mg/kg, 0.51 - 1 mg/kg, 1.01-2.5mg/kg, 2.51 - 5 mg/kg, and >5 mg/kg).

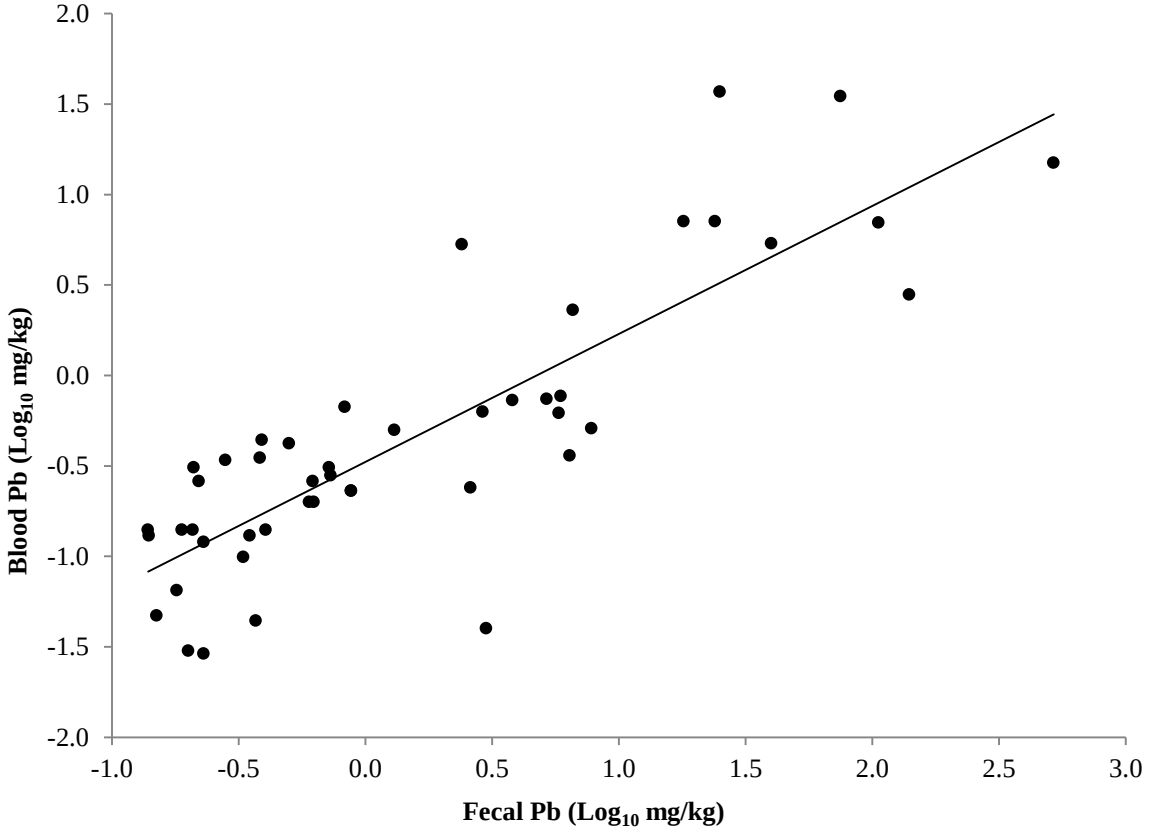

Figure 2. Regression of $\log_{10}$ transformed lead levels from paired fecal samples and blood samples (n=47) collected from rehabilitation Bald Eagles in Iowa in 2012 and 2013. All lead levels were $\log_{10}$ transformed to account for unequal variance between blood lead levels and fecal lead levels and to correct for skewness in the fecal data. Fecal lead levels were a significant predictor of blood lead levels in rehabilitation Bald Eagles ($\beta = 0.707$, $SE = 0.068$, $P < 0.001$, $R^2 = 0.71$). (Pb = lead, mg/kg = milligram/kilogram)

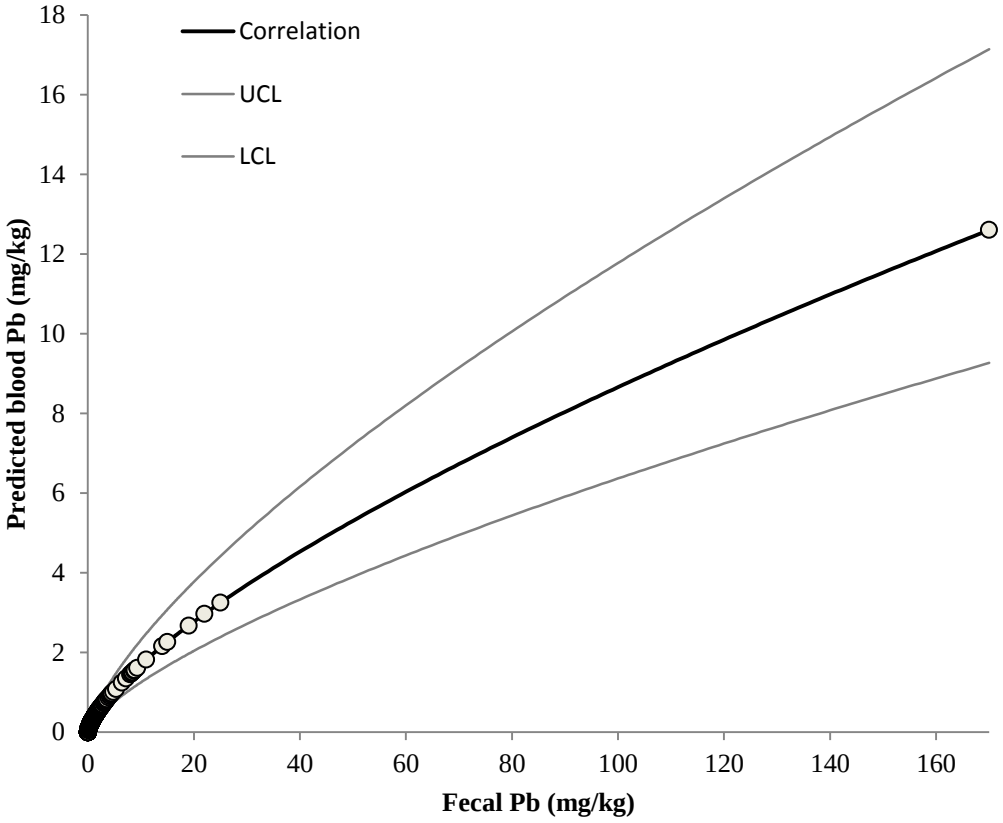

Figure 3. Model of predicted blood lead levels based on fecal lead levels created using a regression model of paired blood and fecal samples (n=47) collected from rehabilitation Bald Eagles in Iowa in 2012 and 2013. The black line represents the estimated mean correlation between blood and fecal lead levels and the gray lines represent the upper and lower bounds of the 95% confidence interval (UCL, LCL). The grey circles represent blood lead estimates of free-flying eagle lead levels based on the fecal lead levels found in free-flying eagles (n=570) in 2012 and 2013. (Pb = lead, mg/kg = milligram/kilogram)

CHAPTER 4. NEST SURVIVAL OF IOWA BALD EAGLES

A paper to be submitted to The Wilson Journal of Ornithology

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ABSTRACT

Measurements of the reproductive success of birds are the most informative when they account for nest-specific attributes, seasonal and yearly patterns, and the possible influence of observer visits. In 2012 and 2013, we studied the nest survival of Bald Eagles (*Haliaeetus leucocephalus*) in Iowa, a species that is sensitive to human disturbance during the nesting cycle. We used Program MARK to model daily nest survival by incorporating covariates accounting for spatial and temporal patterns in survival and specific attributes of each nest. Nest survival was primarily influenced by nest location (survival of nests along the Mississippi River was lower than all other nests), land ownership (survival was higher on public land compared to private land), and proximity to roads (survival was lower near roads). Proximity to water, fecal lead levels, time of nesting season, and year were not statistically significantly related to nest survival. We documented some of the highest nest survival rates reported in Bald Eagles and our results show that our non-invasive sampling method appears to have had minimal impact on the nest survival of this species.

INTRODUCTION

Reproductive success is one of the most important and easily measured aspects in the life history of birds. It is also often used to gauge the overall health of a population or responses to specific conservation or management actions (Nappi and Drapeau 2009, Powell

et al. 2010, Conover et al. 2011, Hovick et al. 2012, Pieron et al 2012, Sexson and Farley 2012, Matthews et al. 2013). There are many approaches to quantifying reproductive success in birds and the methods used are often dictated by the species of interest and the types of data available (Mayfield 1961, Mayfield 1975, Dinsmore et al. 2002, Schmidt et al. 2010, Converse et al. 2013). To minimize disturbance, some birds including nesting raptors are observed remotely and less often than ground nesting birds, because excessive visits by researchers can cause nest failure (Brown et al. 2013). In raptors, this minimalist approach, along with disturbance, can bias estimates of nest success (the proportion of nesting pairs that raise young to fledging age) because detection probabilities are often unequal and because successful pairs are more conspicuous than unsuccessful ones (Steenhof and Kochert 1982, Fuller et al. 1995, Zelenak and Rotella 1997, Martin et al. 2010, Brown and Collopy 2012, McIntyre and Schmidt 2012, Brown et al. 2013).

In the Bald Eagle (*Haliaeetus leucocephalus*), reproductive success can depend upon a variety of factors including food abundance, weather conditions, location, habitat quality, age of adults, number of years holding a nesting territory, size of adult males, environmental contaminants (such as pesticides, heavy metals, and PCBs), and human disturbance (Newman et al 1977, McEwan and Hirth 1979, Swenson et al. 1986, Hansen 1987, Anthony and Isaacs 1989, Grubb and King 1991, Bowerman et al. 1995, Steidl and Anthony 1996, Steidl et al. 1997, Elliot et al. 1998, Millsap et al. 2004, Jenkins and Jackman 2006). In Iowa, Bald Eagles have increased from one nest in 1977 to more than 241 nests in 2013 (Dyar 2010, Shepherd 2013). Yearly surveys conducted by volunteers help to quantify the number of active nests and estimate the number of young fledged each year. In 2013, an estimated 86% of active nests were successful and produced 258 young (Shepherd 2013). While the

number of Bald Eagle nests continues to increase and their nesting range expands, little is known about the factors that influence their reproductive success in Iowa.

Many birds are susceptible to disturbance during key parts of the nesting cycle that can result in nest abandonment (Ellison and Cleary 1978, Tremblay and Ellison 1979, Bolduc and Guillemette 2003, Garrettson et al. 2011). Susceptibility is often species-specific, and for many birds there is scant information to measure the costs of nest disturbance (Westmoreland and Best 1985, Blackmer et al. 2004, Carney and Sydeman 1999, McCarthy and Destefano 2011). In raptors, any human activity that disrupts their normal behavior is considered a disturbance (Richardson and Miller 1997). The Bald Eagle is especially problematic because special permits are required to be in the proximity of eagle nests, yet little is known about their reproductive success in the presence of human disturbance (Mathisen 1968, Grier 1969, Wood and Collopy 1993, Steidl and Anthony 2000). Even when human disturbance is non-invasive, it can adversely affect the distribution and behavior of nesting Bald Eagles resulting in exposure of eggs and nestlings to weather and predators and decreased offspring survival (Newman et al. 1977, Stalmaster and Newman 1978, Grubb and King 1991, McGarigal et al. 1991, Steidl and Anthony 1996, Richardson and Miller 1997, Watson 2004, Watson et al. 1999, Steidl and Anthony 2000). However, other studies have found human disturbance to be negligible in reducing Bald Eagle nest occupancy (whether a nest is occupied or not) and nest success (Mathisen 1968, Fraser et al. 1995, Anthony et al. 1994). In addition, the temporary cooling of eggs that occurs during normal field research, such as nest climbing, may not be a serious problem (Grier 1969, Rosenfield et al. 2007), because Bald Eagles have been shown to have flexible incubation strategies that they can alter depending on weather and human disturbance (Grubb and King 1991, Steidl and Anthony 2000, Watson 2004).

Guidelines have been created for minimizing human disturbance to Bald Eagles, although federal and state regulations are not always consistent. Currently, the Bald Eagle is federally protected under the Lacey Act, the Bald and Golden Eagle Protection Act (Eagle Act), and the Migratory Bird Treaty Act (USFWS 2012a), and in Iowa is officially listed as a Species of Greatest Conservation Need (SCGN), a Species of Special Concern, and is classified as vulnerable (Zohrer 2006). Federal laws prohibit anyone from taking, possessing, or transporting a Bald or Golden Eagle, their parts, nests, or eggs without prior authorization (USFWS 2012a), and the term "take" includes disturbance (USFWS 2010). People engaging in non-motorized recreation, off-road vehicle use, and motorized watercraft use must maintain a distance of ≥100 m (≥200 m in areas with increased visibility) from any Bald Eagle nest during the nesting season (USFWS 2010), while the Iowa Department of Natural Resources encourages people to maintain a distance ≥400 m from wintering and nesting Bald Eagles (Dyar 2010). While these guidelines are useful for the general public, they are prohibitive to eagle researchers who must visit nest sites. In the Midwest, disturbance effects have been reported in a few studies, but not in Iowa where the number of nesting Bald Eagles continues to increase (Shepherd 2013). More quantitative information on the effects of disturbance to Bald Eagle nest survival would better inform guidelines for research-related activities during the nesting season.

In 2012 and 2013, we conducted a nest survival study of Bald Eagles in Iowa by collecting information on nesting, disturbance, and environmental parameters that may be influential to Bald Eagle nest survival. We define nest survival as the probability that a nest will be successful. This study has two primary objectives: 1) to summarize nesting activity to estimate nest initiation, hatch, and fledging dates, and document any research-related

disturbance, and 2) to model the survival of Bald Eagle nests using Program Mark (White and Burnham 1999) and incorporate covariates to understand how spatial, temporal, and other factors influence nest survival (Dinsmore et al. 2002). This study will strengthen our understanding of Bald Eagle nest survival in Iowa and inform future decisions about sampling and monitoring methods to reduce research-related disturbance in Bald Eagle nests.

METHODS

Study species

The Bald Eagle is a year-round resident in Iowa, although their numbers vary seasonally. There are an estimated 200-300 active Bald Eagle nests in the state (Shepherd and Nixon 2011, Shepherd 2013). Bald Eagles nest in Iowa from late January through July, with incubation starting in late February. Eagles occur in the greatest concentrations wherever there is open water and an abundant food source (Kent and Dinsmore 1996, Dyar 2010). Bald Eagle nests are most heavily concentrated in northeastern part of the state. Bald Eagles also nest along river corridors throughout the remainder of Iowa, but at lower densities, with the fewest nests occurring in western Iowa (Iowa DNR 2011, Iowa DNR 2012).

Study area

We randomly selected 110 Bald Eagle nests from a list of more than 200 nests classified as active by the Iowa Department of Natural Resources and the United States Fish and Wildlife Service in 2011 (Iowa DNR 2011). Nearly half of these nests were in the Upper Mississippi River National Fish and Wildlife Refuge in northeastern Iowa, while the remaining nests were scattered throughout the rest of Iowa on both private and public lands.

Nests sampled in 2012 were resampled in 2013 and any nests that were found abandoned, destroyed, or inaccessible in 2013 were replaced with randomly chosen backup nests.

Sampling

Nest sampling was spatially and temporally stratified to enable us to test two specific hypotheses about lead exposure in Iowa (Lead exposure in free-flying Bald Eagles in Iowa; see Chapter 2). First, nests were spatially stratified into a Mississippi River group and a non-Mississippi River group. These two groups were sampled differently, because of where their nests occurred. Nearly all nests in the Mississippi River group were located on islands and had to be approached by boat (either airboat or outboard motorboat), while all nests in the non-Mississippi River group were approached on foot because they occurred on public and private lands that were easily accessible from nearby roads. We also stratified nests temporally by sampling each nest site once during the winter and once during the spring. Visiting each nest twice during the nesting cycle allowed us to record nesting activity and nestling age, document nest abandonment, and estimate nest initiation, hatch, and fledging dates, all of which was necessary to model nest survival.

We visited nest sites from 16 February to 29 March 2012 (winter) and from 4 May to 22 May 2012 (spring). In 2013, we visited nest sites from 20 February to 28 March (winter) and from 1 May to 3 June (spring). The first visits occurred during the incubation period and the second visits occurred when the Bald Eagle chicks were 3-9 weeks old. These visit periods were mandated by the USFWS and were perceived to be the least risky times for causing abandonment or negative impacts for nestlings. We did not monitor nests from clutch initiation to fledge. Instead our results pertain to the onset of incubation, defined as the day the first egg was laid (USFWS 2007, CCB 2013), up to the point nestlings were between 3-9

weeks old (98 days maximum). Prior to approaching each nest, we would confirm either incubation in the winter or nest activity in the spring using binoculars or spotting scopes. We spent up to 30 minutes within 100 m of each nest site. During winter visits 2 adults were present at the nest incubating eggs, whereas during spring 2 adults were present along with 1-3 nestlings if the nest was active. We collected fecal samples for lead testing from below nesting Bald Eagles using non-invasive collection techniques to reduce sampling-associated disturbance (Rosenfield et al. 2007).

Monitoring nesting eagles

During winter sampling we returned to 21 nests in 2012 and 20 nests in 2013 within 24-72 hours after sampling to document any nest abandonment possibly related to our sampling. We determined occupancy status, number of adults present, and adult behavior. Incubation was assumed if one adult was observed sitting down flat and low in the nest, as if on eggs. We also collected data to document the short-term and long-term disturbance associated with our presence. To document disturbance associated with each visit to a nest, we made observations (when possible) on Bald Eagle behavior during and immediately after the visit, such as whether the adult(s) flushed or if they seemed agitated by our presence, and also the amount of time it took an adult to return to the nest.

Nest survival modeling

During the spring sampling session in 2012 and 2013 we attempted to count, photograph, and age the nestlings at active nest sites, in addition to documenting the adult behavior and time to return to the nest. Nestling plumage was visually assessed to estimate the age of nestlings to approximate number of weeks old (Carpenter 1989). Using the weekly age data, we then estimated nest initiation, hatching, and fledging dates. For our estimates,

we converted age (in weeks) to days by taking the number of days in a given week of age and subtracting 3 days to get an average # of days (# days in weeks - 3 days). Our estimates of nest initiation and hatching dates were based upon the mean reported incubation period for Bald Eagles (35 days; USFWS 2007) and our estimates of fledging dates were based on the mean reported fledging age of 11 weeks (USFWS 2012b). Because nest monitoring was limited to two visits per nest, we never followed them to fledging and instead our inferences pertain to nests that reached 9 weeks of age. In addition, we were unable to model survival separately for the incubation and nestling stages (e.g., for failed nests we had no way to assign exposure days to each stage) and assumed there were no stage-specific differences in survival.

We then created models in program MARK (White and Burnham 1999) using the nest survival model (Dinsmore et al. 2002). Because we were focused on collecting samples for lead testing during the limited duration of our visits, we were not able to collect any nest-site measurements. Instead, we chose to model nest survival based upon location (e.g., Mississippi River vs. non-Mississippi River, land ownership, proximity to nearest water and road), reported fecal lead levels, linear effect of day within nesting season (T), and year.

We hypothesized that Mississippi River nests would have lower daily survival rates (DSR) compared to non-Mississippi River nests because Mississippi River nests would be subject to higher levels of human disturbance (due to increased human presence for commercial and recreational purposes [USGS 2013, Mundahl et al. 2013]), than nests away from the Mississippi River. Conversely, we hypothesized that closer proximity to water would have a positive effect on DSR, because of increased availability of food (fish and waterfowl). We also hypothesized that nests on private lands would have higher DSR

compared to nests on public lands, due to lower levels of human disturbance on private lands. We also hypothesized that nests closer to roads would have lower DSR, and nests with lower fecal lead levels would have higher DSR. By quantifying the probability of nest survival in relation to the above covariates, we attempted to characterize the magnitude of influence that each covariate had on Bald Eagle nest survival in Iowa. Models were ranked using the corrected Akaike's Information Criterion (AICc, Akaike 1973). For important model effects we report the coefficient, its standard error (SE), and the 95% confidence limits (CL). We also summarized information on nest initiation dates (date first egg was laid; USFWS 2007, CCB 2013) and hatching dates (date first egg hatched; USFWS 2007, CCB 2013).

RESULTS

We visited 110 nests in 2012 and 107 nests in 2013 (Figure 1). During spring 2012 sampling visits we found 91 active nests, 13 inactive nests, and the status of 6 nests was undetermined (Table 1). During spring 2013 sampling visits, we found 89 active nests, 15 inactive nests, and the status of 3 nests was undetermined (Table 1). All nests with undetermined status had conflicting evidence that made it difficult to determine the activity level. In some cases, young could not be seen, but adults were perched in nearby trees. However, the adults were not agitated by our presence, and we found little to no feces under the nest tree. In other cases, young were not visible and no adults were nearby, but feces were found below the nest tree.

Nest survival modeling

The daily survival of Bald Eagle nests was most influenced by location (relative to the Mississippi River), whether it was on public or private land, and its proximity to roads (Table 2). Nests on the Mississippi River had lower daily survival than nests away from the

Mississippi River ($\beta_{Mississippi}$ = -2.08, SE = 0.54, 95% CLs were -3.14, -1.02 from the best model). In addition, nests on public land had higher daily survival than nests on private land (β_{Public} = 1.16, SE = 0.84, 95% CLs were -0.55, 2.76) and nests closer to roads had a slightly lower daily survival than nests further from roads (β_{Roads} = -0.00015, SE = 0.00019, 95% CLs were -0.00053, -0.00021). There was also weak evidence for a linear decline in nest survival across the nesting season (β_{T} = -0.00036), although the 95% confidence interval for this effect included zero. None of the other covariates, including levels of lead during winter and spring nest visits, were statistically significant.

Estimated nest initiation, hatching, and fledging dates

During 2012 and 2013, we estimated that nest initiation dates for Bald Eagle nests found active in the spring ranged from 19 February to 21 March, with a mean estimated nest initiation date of 4 March. Estimated hatch dates for Bald Eagle nests found active in the spring ranged from 26 March to 25 April (mean = 8 April) while estimated fledging dates ranged from 11 June to 11 July (mean = 24 June).

Short-term Monitoring

In 2012, we monitored 21 nests within 24-72 hours after winter sampling. In four cases, monitoring occurred outside the 24-72 hour window due to weather and other logistical reasons. Regardless, adults were observed incubating at 19 of the 21 nests, standing on the nest at 1 site, and perched in a nearby tree at the remaining nest. In 2013, we monitored 20 nests within 24-72 hours after our winter visits and found adults actively incubating at all 20 nests. All monitored nests were revisited during spring sampling each year. In 2012, we found 18 of the 21 nests active with nestlings, one nest was inactive, and

the status of two nests could not be determined. In 2013, we found 19 of the 20 nests active with nestlings and one nest was inactive.

Nest survival predictions

We estimated the overall nest survival probability for Mississippi River and non-Mississippi River nests on private and public land (Table 3), using the model that included covariates for location (relative to the Mississippi River), whether it was on public or private land, and its proximity to roads {S(Mississippi vs. non-Mississippi + public vs. private + roads); ΔAICc = 1.25}. We justified the use of this model for predictions because it contained all of the statistically significant model effects and was a highly competitive model. Our results pertain to period of time that includes the onset of incubation up to nestlings at 9 weeks of age (98 days total) and do not include the last 1-2 weeks needed to reach fledging age. Estimated survival was greatest for non-Mississippi River nests on public land (0.98, 95% CL [0.88, 1.00]) and private land (0.92, 95% CL [0.80, 0.97]). Estimated survival was lower for Mississippi River nests on public land (0.67, 95% CL [0.52, 0.79]) and lowest for Mississippi River nests on private land (0.19, 95% CL [0.00, 0.74]).

DISCUSSION

In this study we provide the first assessment of factors influencing Bald Eagle nest survival in Iowa. This is a timely study because this species has increased from one nest in 1977 to more than 241 nests in 2013 (Dyar 2010, Shepherd 2013). Our work documented that Iowa Bald Eagle nests have high survival rates (for the timeframe that we measured), that survival differs between nests on the Mississippi River and those elsewhere in Iowa, and that land ownership and the proximity to roads may be important predictors of nest survival. Below, we compare our main findings with others in the published literature and use this

information to document that our research-related disturbance appears to have had minimal impact on nests of this species.

In other studies that have reported Bald Eagle reproductive success, different methods and terminologies are employed. For example, many studies quantify mean productivity as a measure of reproductive success and this is usually presented as the number of young per successful nest or the number of young produced per occupied territory (Mathisen 1968, Grier 1969, McEwan and Hirth 1979, Swenson et al. 1986, Anthony et al. 1994, Steidl et al. 1997, Elliot et al 1998, Jenkins and Jackman 2006). Nest success estimates are also reported in different ways, with some studies reporting the number of years a nest is occupied divided by the number of years young are produced (Jenkins and Jackman 2006), while others report the percent of pairs or occupied sites that producing one or more young to fledging age (Swenson et al. 1986, Anthony et al. 1994, Steidl et al. 1997). Depsite differences in these methods between studies and regions, comparisons can still can be made. For example Anthony et al. (1994) found that 60% of occupied nests produced young in Oregon, which was similar to Swenson et al. (1986) who also reported that 60% of occupied nests were successful in the Greater Yellowstone region. Jenkins and Jackman (2006) found that mean nesting success in Northern California was 62%, while Steidl et al. (1997) compared two river basins in Alaksa and found that nest success was 59% and 48%. Mathisen also compared the nest success between three groups in Minnesota and found that nests were successful 48%, 54%, and 57% of the time. More recently, Mundahl et al. (2013) reported Bald Eagle nest success rates ranging from 38% up to 92% in four pools in the Upper Mississippi National Fish and Wildlife Refuge, north of where we conducted our Mississippi

River nest sampling. All of the above studies utilized apparent nest success (proportion of successful nests in a sample), which is biased high relative to true survival (Mayfield 1961).

As mentioned earlier, we did not monitor nests from clutch initiation to fledge. Instead we modeled nest survival from the onset of incubation to when nestlings were 9 weeks old (98 days total), which is shorter than the typical fledging age of 10-12 weeks. Broley (1947) and Anthony et al. (1994) documented that nestling mortality was a small fraction of the total nest failures in Bald Eagles in Florida (2%) and Oregon (3%), suggesting that when food is abundant and fratricide is low, nestling mortality should also be low. Compared to the above studies, we had an apparent nest success of 83% for 2012-13 combined, although this estimate would be slightly lower if calculated to the full 10-12 weeks of age needed for fledging. When modeling nest survival using Program Mark we found variation as a function of location and land use. Specifically, non-Mississippi River nests had survival probabilities of 0.98 for nests on public land and 0.92 for nests on private land. However, for Mississippi River nests, the survival probabilities were 0.67 for nests on public land and 0.19 for nests on private land (note: there were only two nests in this category).

One explanation for the higher survival in non-Mississippi River nests could be that the overall levels of human activity between Mississippi River nests and non-Mississippi nests are different. The Upper Mississippi National Fish and Wildlife Refuge, where nearly all of the Mississippi river nests were located, is not only a refuge for wildlife, but it is also a popular recreation area with roughly 1.5 million visitors per year (USGS 2013). These higher levels of human activity could feasibly cause greater levels of human disturbance at these Bald Eagle nests compared to non-Mississippi River nests. It has also been reported that Bald

Eagle pairs are more likely to build alternate nests in areas of greater human disturbance (Newman et al 1977, Anthony and Isaacs 1989). Anecdotally, we did observe more nesting eagle pairs on the Mississippi River which created and used alternate nests within their territory during the second year of sampling compared to pairs at non-Mississippi River nests. Some studies have also shown that Bald Eagles can adapt to higher levels of human disturbance in areas of increased human activity, without their reproductive success being negatively impacted (Mathisen 1968, Fraser et al. 1995, Anthony et al. 1994), while other studies suggest that human disturbance can adversely affect the distribution and behavior of nesting Bald Eagles resulting in decreased offspring survival (Newman et al. 1977, Stalmaster and Newman 1978, Grubb and King 1991, McGarigal et al. 1991, Steidl and Anthony 1996, Richardson and Miller 1997, Watson 2004, Watson et al. 1999, Steidl and Anthony 2000).

Another possible explanation for lower nest survival of Mississippi River nests could be due to increased intraspecific competition, because the density of nesting and nonbreeding Bald Eagles is much greater on the refuge than elsewhere in Iowa. This intraspecific competition could impact reproductive success in years when food supplies are less abundant (Hansen 1987, Steidl et al. 1997). This is consistent with Mundahl et al. (2013), who reported that successful nests in the Upper Mississippi National Fish and Wildlife Refuge tended to be located significantly further from other active nests than were unsuccessful nests. Nests located away from Mississippi River would likely face even less competition in comparison.

The differences between Mississippi River nests and non-Mississippi River nests might also be the result of differences in our approach when sampling nests. Mississippi River nests were approached by boat and then on foot, while non-Mississippi River nests

were only approached on foot. However, two previous studies found that pedestrian traffic was the most impactful type of human disturbance (Watson 2004, Grubb and King 1991), greater than boat traffic, so we do not think the difference in how we approached each nest were important because all nests were subject to researchers walking around the base of the nest tree when collecting fecal samples.

We also found that daily survival of Bald Eagle nests was lower on private lands compared to public lands and that proximity to roads had a slightly negative influence on nest survival. Mundahl et al. (2013) reported that human disturbance (including traffic from highways, railroads, commercial barges, and recreational boats) did not appear to be limiting the increase of nesting Bald Eagles in the Upper Mississippi National Fish and Wildlife Refuge. McEwan and Hirth (1979) also reported that mean production of young eagles in Florida had no correlation with proximity to roads or proximity to water, while Mundahl et al. (2013) did notice a strong tendancy for successful nests to be located further from water. Grubb and King (1991) reported that proximity to vehicles did cause disturbance, such as flushing, but generally that pertained to off-road vehicles. Perhaps nests on private lands are visited more often by landowners because of the novelty and ease of access compared to public lands. Anecdotally, we did speak with some private landowners who routinely placed the carcasses of deer or livestock below the nest on their land to supplement the food of the resident nesting pair. Perhaps nests on private lands are less accustomed to human disturbance, because they haven't been habituated to the recreational and commercial activites that may occur on well established public lands. Or perhaps human activity on public lands (other than the refuge) is simply lower during the nesting period. We did not

find not find any statistical difference between the proximity to roads for nests on public versus private lands (P>0.05).

Despite the fact that we did not follow nestlings to fledging, this study improves our understanding of nest survival and other vital nesting parameters for Bald Eagles in Iowa. Our results for short-term nest monitoring (24-72 hours after winter sampling) showed that all adults remained at their nest sites and that the majority of birds were observed incubating during our nest observations (95% of monitored nests). This suggests that our sampling visits of up to 30 minutes within 100 m of the nest had little to no short-term impact on the behavior of incubating adults and caused no known cases of nest abandonment. Overall we documented some of the highest nest survival rates reported for Bald Eagles and found that nest survival was lower for nests along the Mississippi River, higher for nests on public land, and slightly lower when nests were closer to roads. We further infer that our non-invasive sampling activity below nests had minimal impact on the nest survival of this species.

LITERATURE CITED

Akaike, H. 1973. Information theory and an extension of the maximum likelihood principle. Pages 267-281 *in* International Symposium on Information Theory, 2nd, Tsahkadsor, Armenian SSR.

Anthony, R.G., and F.B. Isaacs. 1989. Characteristics of bald eagle nest sites in Oregon. Journal of Wildlife Management 53:148-159.

Anthony, R.G., R. W. Frenzel, F.B. Isaacs, and M. G. Garrett. 1994. Probable causes of nesting failures in Oregon's bald eagle population. Wildlife Society Bulletin 22:576–582.

Blackmer, A.L., J.T. Ackerman, and G.A. Nevitt. 2004. Effects of investigator disturbance on hatching success and nest-site fidelity in a long-lived seabird, Leach's storm-petrel. Biological Conservation 116:141-148.

Bolduc, F., and M. Guillemette. 2003. Human disturbance and nesting success of common eiders: interaction between visitors and gulls. Biological Conservation 110:77-83.

Broley, C.L. 1947. Migration and nesting of Florida bald eagles. Wilson Bull. 59:3-20.

Bowerman, W.W., J.P. Giesy, D.A. Best, and V.J. Kramer. 1995. A review of factors affecting productivity of bald eagles in the Great Lakes region: Implications for Recovery. Environmental Health Perspectives 103:51-59.

Brown, J.L., and M.W. Collopy. 2012. Bayesian hierarchical model assessment of nest site and landscape effects on nest survival of aplomado falcons. Journal of Wildlife Management 76:800-812.

Brown, J.L., K. Steenhof, M.N. Kochert, and L. Bond. 2013. Estimating raptor nesting success: old and new approaches. Journal of Wildlife Management 77:1067-1074.

Carney, K.M., and W.J. Sydeman. 1999. A review of human disturbance effects on nesting colonial waterbirds. Waterbirds: The International Journal of Waterbird Biology 22:68-79.

Carpenter, G. 1989. An illustrated guide to identifying developmental stages of bald eagle nestlings. Draft. San Francisco Zoological Society.

Conover, R.R., S.J. Dinsmore, and L.W. Burger Jr. 2011. Effects of conservation practices on bird nest density and survival in intensive agriculture. Agriculture, Ecosystems and Environment 141:126-132.

Converse., S.J., J.A. Royle, P.H. Adler, R.P. Urbanek, and J.A. Barzen. 2013. A hierarchical nest survival model integrating incomplete temporally varying covariates. Ecology and Evolution 3:4439-4447.

Dinsmore, S. J., G. C. White, and F. L. Knopf. 2002. Advanced techniques for modeling avian nest survival. Ecology. 83:3476-3488.

Dyar, J. 2010. Bald eagles in Iowa. Iowa Department of Natural Resources.

Elliot, J.E., I.E. Moul, and K.M. Cheng. 1998. Variable Reproductive Success of Bald Eagles on the British Columbia Coast. Journal of Wildlife Management 62:518-529.

Ellison, L.N., and L. Cleary. 1978. Effects of human disturbance on breeding of double-crested cormorants. The Auk 95:510-517.

Fraser, J.D., L.D. Frenzel, and J.E. Mathisen. 1985. The impact of human activities on breeding bald eagles in North-Central Minnesota. Journal of Wildlife Management 49:585-592.

Fuller, M.R., J.S. Hatfield, and E.L. Lindquist. 1995. Assessing Ground-Based Counts of Nestling Bald Eagles in Northeastern Minnesota. Wildlife Society Bulletin 23:169-174.

Garrettson, P.R., K.D. Richkus, F.C. Rohwer, and W.P. Johnson. 2011. Factors influencing investigator-caused nest abandonment by North American dabbling ducks. Canadian Journal of Zoology 89:69-78.

Grier, J.W. 1969. Bald eagle behavior and productivity responses to climbing nests. Journal of Wildlife Management 33:961-966.

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.

Hansen, A. J. 1987. Regulation of bald eagle reproduction rates in Southeast Alaska. Ecology 68:1387-1392.

Hovick, T.J., J.R. Miller, S.J. Dinsmore, D.M. Engle, D.M. Debinski, and S.D. Fuhlendorf. 2012. Effects of fire and grazing on grasshopper sparrow nest survival. Journal of Wildlife Management 76:19-27.

Jenkins, J.M., and R.E. Jackman. 2006. Lifetime Reproductive Success of Bald Eagles in Northern California. The Condor 108:730-735.

Martin, J.M., L.C. Branch, R.N. Raid, and S.C. Beyeler. 2010. Temporal instability of agricultural habitat reduces reproductive success of barn owls (*Tyto alba*). The Auk 127:909-916.

Matthews, T.W., A.J. Tyre, J.S. Taylor, J.J. Lusk, L.A. Powell. 2013. Greater prairie-chicken nest success and habitat selection in Southeastern Nebraska. Journal of Wildlife Management 77:1202-1212.

Mathisen, J. E. 1968. Effects of human disturbance on nesting of bald eagles. Journal of Wildlife Management 32:1-6.

Mayfield, H. R. 1961. Nesting success calculated from exposure. Wilson Bulletin 73:255-261.

Mayfield, H. R. 1975. Suggestions for calculating nest success. Wilson Bulletin 87:456-466.

McCarthy, K.P., and S. Destefano. 2011. Effects of spatial disturbance on common loon nest site selection and territory success. Journal of Wildlife Management 75:289-296.

McEwan, L.C., and D.H. Hirth. 1979. Southern bald eagle productivity and nest site selection. Journal of Wildlife Management 43:585-594.

McGarigal, K., R.G. Anthony, and F.B. Isaacs. 1991. Interactions of humans and bald eagles on the Columbia River estuary. Wildlife Monographs 115:3-47.

McIntyre, C. L., and J. H. Schmidt. 2012. Ecological and environmental correlates of territory occupancy and breeding performance of migratory golden eagles *Aquila chrysaetos* in interior Alaska. Ibis 154:124–135.

Millsap, B., T. Breen, E. McConnell, T. Steffer, L. Phillips, N. Douglas, and S. Taylor. 2004. Comparative fecundity and survival of bald eagles fledged from suburban and rural natal areas in Florida. Journal of Wildlife Management 68:1018-1031.

Mundahl, N.D., A.G. Bilyeu, and L. Maas. 2013. Bald eagle nesting habitats in the Upper Mississippi River National Wildlife and Fish Refuge. Journal of Fish and Wildlife Management 4(2): 362-376; e1944-687X. doi: 10.3996/012012-JFWM-009.

Nappi, A., and P. Drapeau. 2009. Reproductive success of the black-backed woodpecker (*Picoides arcticus*) in burned boreal forests: Are burns source habitats? Biological Conservation 142:1381-1391.

Newman, J.R., W.H. Brennan, and L.M. Smith. 1977. Twelve-year changes in nesting patterns of bald eagles (*Haliaeetus leucocephalus*) on San Juan Island, Washington. The Murrelet 58:37-39.

Pieron, M.R., M.J.K. Darr, and F.C. Rohwer. 2012. Duck nest success adjacent to predator-reduced sites. Journal of Wildlife Management 76:1450-1455.

Powell, L.L., T.P. Hodgman, W.E. Glanz, J.D. Osenton, and C.M. Fisher. 2010. Nest-site selection and nest survival of the rusty blackbird: does timber management adjacent to wetlands create ecological traps. The Condor 112:800-809.

Rosenfield, R. N., J. W. Grier, and R. W. Fyfe. 2007. Reducing management and research disturbance. Pages 351-364 in Raptor Research and Management Techniques. Hancock House Publishers. Surrey, Canada.

Richardson, C. T., and C. K. Miller. 1997. Recommendations for protecting raptors from human disturbance: a review. Wildlife Society Bulletin 25:634-638.

Schmidt, J.H., J.A. Walker, M.S. Lindberg, D.S. Johnson, and S.E. Stephens. 2010. A general bayesian hierarchical model for estimating survival of nests and young. The Auk 127: 379-386.

Sexson, M.G., and G.H Farley. 2012. Snowy plover nest survival in Kansas and effective management to counter negative effects of precipitation. Journal of Wildlife Management 76: 1587-1596.

Shepherd, S. and B. Nixon. 2011. Iowa's 2011 midwinter bald eagle survey report. Iowa Department of Natural Resources.

Shepherd, S. 2013. Bald eagle (*Haliaeetus leucocephalus*) nesting in Iowa 2013. Iowa Department of Natural Resources. <http://www.iowadnr.gov/Portals/idnr/uploads/wildlife/research/BENestSummary_2012.pdf>. Accessed 28 January 2014.

Stalmaster, M. V., and J. R. Newman. 1978. Behavioral responses of wintering bald eagles to human activity. Journal of Wildlife Management 42:506-513.

Steenhof, K., and M.N. Kochert. 1982. An evaluation of methods used to estimate raptor nesting success. Journal of Wildlife Management 46:885-893.

Steidl, R. J., and R.G. Anthony. 1996. Responses of bald eagles to human activity during the summer in Interior Alaska. Ecological Applications 6:482-491.

Steidl, R. J., K.D. Kozie, and R.G. Anthony. 1997. Reproductive Success of Bald Eagles in Interior Alaska. Journal of Wildlife Management 61:1313-1321.

Steidl, R. J., and R.G. Anthony. 2000. Experimental effects of human activity on breeding bald eagles. Ecological Applications 10:258-268.

Swenson, J.E., K.L. Alt, R.L. Eng. 1986. Ecology of bald eagles in the Greater Yellowstone ecosystem. Wildlife Monographs 95:3-46.

The Center for Conservation Biology. (CCB) 2013. Facts about Bald Eagles. <http://www.ccbbirds.org/what-we-do/research/species-of-concern/virginia-eagles/facts-about-eagles/>. Accessed 30 May 2012.

Tremblay, J., and L.N. Ellison. 1979. Effects of human disturbance on breeding of black-crowned night herons. The Auk 96:364-369.

United States Fish and Wildlife Service (USFWS). 2007. Bald eagle fact sheet: natural history, ecology, and history of recovery. <http://www.fws.gov/midwest/eagle/recovery/biologue.html>. Accessed 30 May 2012.

United States Fish and Wildlife Service (USFWS). 2010. Guidelines to avoid disturbing bald eagles. Bald Eagle management guidelines and conservation measures. <http://www.fws.gov/midwest/eagle/guidelines/index.html>. Accessed 30 May 2012.

United States Fish and Wildlife Service (USFWS). 2012a. Federal laws that protect bald eagles. <http://www.fws.gov/midwest/eagle/protect/laws.html>. Accessed 30 May 2012.

United States Fish and Wildlife Service (USFWS). 2012b. Bald eagle natural history and sensitivity to human activity information.

<http://www.fws.gov/midwest/eagle/recovery/biologue.html>. Accessed 30 May 2012.

United States Geological Survey (USGS). 2013. National Wildlife Refuge Visitor Survey 2012: Individual Refuge Results for La Crosse District, Upper Mississippi River National Wildlife and Fish Refuge. No. DS-754.

Watson, J. M., D.J. Pierce, and B.C. Cunningham. 1999. An active Bald Eagle nest associated with unusually close human activity. Northwestern Naturalist 80:71-74.

Watson, J. M. 2004. Responses of nesting bald eagles to experimental pedestrian activity. The Journal of Raptor Research 38:295-303.

Westmoreland, D., and L.B. Best. 1985. The effect of disturbance on mourning dove nesting success. The Auk 102:774-780.

White, G.C., and K.P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study 46:120-139.

White, C. M., and T. L. Thurow. 1985. Reproduction of ferruginous hawks exposed to controlled disturbance. The Condor 87:14-21.

Wood, P.B., and M.W. Collopy 1993. Effects of egg removal on bald eagle productivity in Northern Florida. Journal of Wildlife Management 57:1-9.

Zelenak, J.R., and J.J Rotella. 1997. Nest success and productivity of ferruginous hawks in northern Montana. Canadian Journal of Zoology 75:1035-1041.

Zohrer, J.J. 2006. The Iowa comprehensive wildlife action plan. Iowa Department of Natural Resources, Des Moines, IA.

TABLES

Table 1. Summary of Bald Eagle nest status during spring visits in Iowa, 2012 and 2013. Active nests had nestlings and/or adults present on the nest at the time of sampling. Nests with unknown status were inaccessible or had feces below the nest, but adult and/or nestlings were not observed; or little or no feces were present though adults were observed in the vicinity. Inactive nests had blown down or had no adults or nestlings present at the time of sampling and little or no feces were present.

Nest status	2012 nests	%	2013 nests	%
Active	91	82.7%	89	83.1%
Unknown	6	5.5%	3	2.8%
Inactive	13	11.8%	15	14.0%
TOTAL	110	100%	107	100%

Table 2. Models of daily survival rate for Bald Eagle nests in Iowa monitored from 16 February to 22 May 2012 and from 20 February to 3 June 2013. Models are listed in descending order by AICc weight. Models were created in Program MARK using the following covariates: Mississippi River vs. non-Mississippi River (Mississippi vs. non-Mississippi), public vs. private land (public vs. private), proximity to nearest water (water), proximity to nearest road (roads), reported fecal lead levels (winter lead and spring lead), the linear effect of day within nesting season (T), and a model with constant daily nest survival (no effects).

Effect(s) on nest survival	[1]ΔAICc	Weight	K	Deviance
Mississippi vs. non-Mississippi	0	0.26	2	146.46
Mississippi vs. non-Mississippi + public vs. private	0.51	0.20	3	144.97
Mississippi vs. non-Mississippi + public vs. private + roads	1.25	0.14	4	143.71
Mississippi vs. non-Mississippi + roads	1.32	0.13	3	145.78
Mississippi vs. non-Mississippi + T	2.00	0.09	3	146.46
Mississippi vs. non-Mississippi + public vs. private + T	2.47	0.07	4	144.93
Mississippi vs. non-Mississippi + public vs. private + roads +T	3.22	0.05	5	143.68
Mississippi vs. non-Mississippi + roads + T	3.33	0.05	4	145.78
public vs. private + roads	9.98	0.00	3	154.44
public vs. private + roads + T	10.73	0.00	4	153.19
roads + T	11.14	0.00	3	155.60
public vs. private	11.42	0.00	2	157.89
roads	11.58	0.00	2	158.04
public vs. private + T	11.60	0.00	3	156.06
T	16.88	0.00	2	163.34
no effects	20.11	0.00	1	168.57
winter lead	20.89	0.00	2	167.35
water	21.88	0.00	2	168.34
year	21.92	0.00	2	168.38
spring lead	21.96	0.00	2	168.42
winter lead + spring lead	22.75	0.00	3	167.21

[1]The AICc value for the best model was 150.46.

Table 3. Nest survival rate estimates for Bald Eagle nests in Iowa monitored from 16 February to 22 May 2012 and from 20 February to 3 June 2013. This rate is the overall probability of surviving from onset of incubation to age 9 weeks (total of 98 days). Estimates were derived from the model that included covariates for location (relative to the Mississippi River), whether it was on public or private land, and its proximity to roads {S(Mississippi vs. non-Mississippi + public vs. private + roads)}. N is the number of unique nests in each group with sufficient data to be included in our nest survival analyses. LCL is the 95% lower confidence limit and UCL is the 95% upper confidence limit.

Group	Estimate	95% LCL	95% UCL	N
Mississippi River (public)	0.67	0.52	0.79	58
Mississippi River (private)	0.19	0.00	0.74	2
non-Mississippi River (public)	0.98	0.88	1.00	11
non-Mississippi River (private)	0.92	0.80	0.97	46

FIGURES

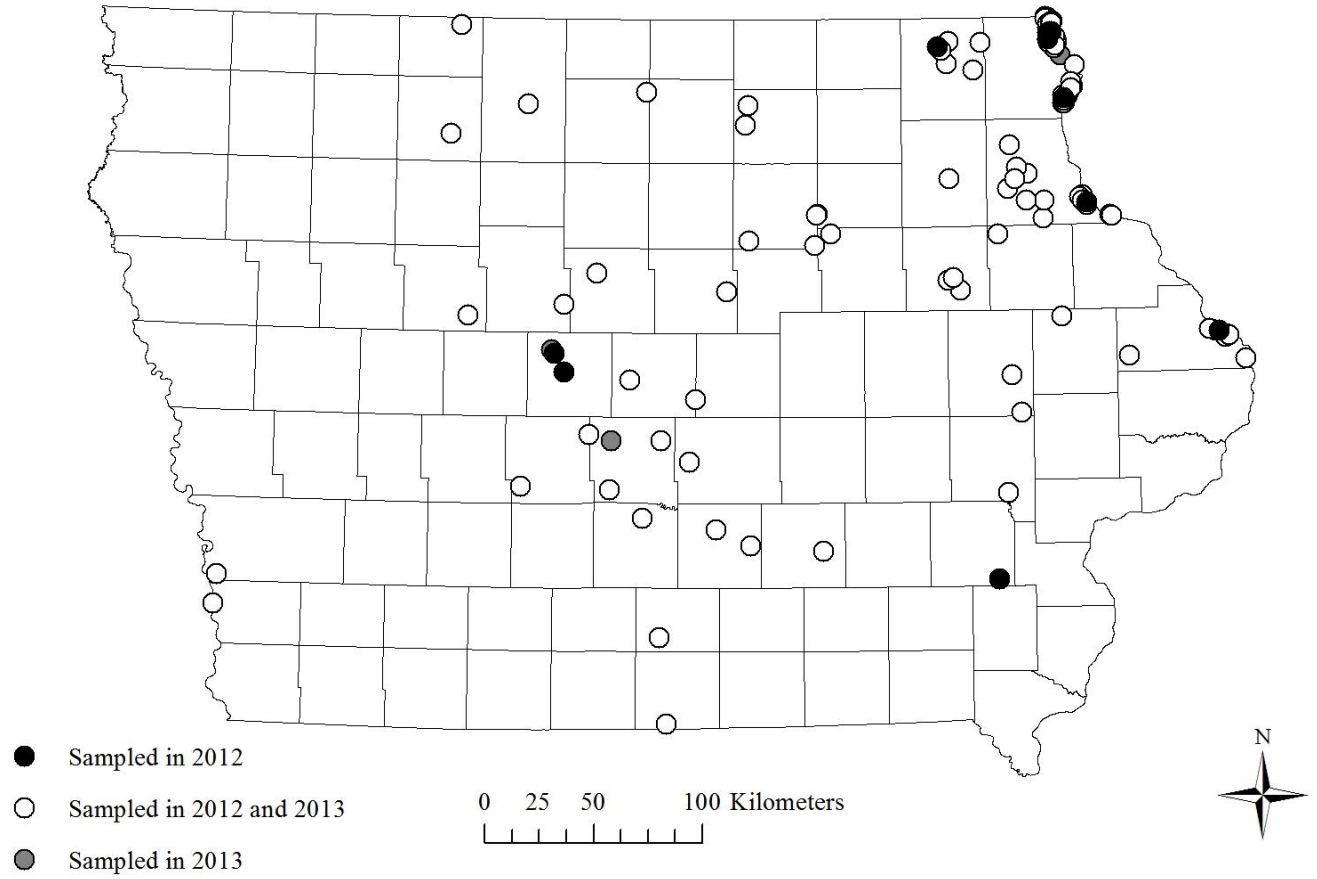

Figure 1. Map of Iowa showing the location of 110 Bald Eagle nests below which feces were collected in 2012 (n=110) and 2013 (n=107). Black circles represent nests only sampled in 2012, white circles represent nests sampled in both 2012 and 2013, and gray circles represent nests only sampled in 2013. Nests were chosen randomly from a list of more than 200 nests considered active in 2011 by the U.S. Fish and Wildlife Service and the Iowa Department of Natural Resources. At the beginning of both the 2012 and 2013 field seasons, nests found to be inactive, destroyed, or inaccessible were replaced with randomly selected backup nests.

CHAPTER 5. GENERAL CONCLUSIONS AND RECOMMENDATIONS

In 2012 and 2013, I investigated temporal and spatial patterns in dietary lead exposure in wintering and nesting Bald Eagles (*Haliaeetus leucocephalus*) in Iowa by collecting fecal samples from the base of roost and nest trees in 2012 and 2013. During the same time period, I partnered with professional wildlife rehabilitators, who simultaneously collected blood and fecal samples from Bald Eagles as they were admitted to one of three wildlife rehabilitation centers in Iowa. In 2012 and 2013, I also conducted a nest survival study of Bald Eagles in Iowa by collecting nesting information, and disturbance and environmental parameters that may be influential to Bald Eagle nest survival. We used Program MARK to model daily nest survival by incorporating covariates accounting for spatial and temporal patterns in survival and specific attributes of each nest. The major findings of our non-invasive investigation of Bald Eagles in Iowa are summarized below:

1) Our study documented detectable amounts of lead in 93% of feces collected from free-flying eagles. However, we found that the magnitude of lead exposure in nesting and wintering Bald Eagles in Iowa was low in the majority of cases and similar to lead levels typically found in the environment. Our results were also similar to fecal lead levels found in other bird species inhabiting known non-lead contaminated sites (Fitzner et al. 1995, Dauwe et al. 2000, Ek et al. 2004, Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010). Fecal lead levels from nests sampled during the winter were higher than nests sampled in the spring, fecal lead levels from non-Mississippi River nests were higher than Mississippi River nests, and fecal lead levels from nesting eagles were higher than wintering eagles, but these differences were not statistically significant. We found no statistical difference between fecal

lead levels from nest sites on public versus private land and no correlation between lead exposure and land ownership, watershed, proximity to water, proximity to potential environmental sources of lead, or number of Iowa deer harvested or lost due to wounding. Our results indicate that the majority of free-flying nesting and wintering Bald Eagles in Iowa experience low levels of lead exposure.

2) In comparison to free-flying Bald Eagles in Iowa, we documented lead in 100% of the feces collected from rehabilitation Bald Eagles. We found that fecal lead levels in rehabilitation Bald Eagles were significantly higher than lead levels in free-flying Bald Eagles. We also compared blood lead levels with fecal lead levels taken from rehabilitation Bald Eagles and found that fecal lead levels were higher than blood lead levels, but these differences were not statistically significant. Our results suggest that lead levels in rehabilitation Bald Eagles are not representative of lead exposure levels in free-flying Bald Eagles, but rather representative of a small subset of the population.

3) Using linear regression, we examined the relationship between fecal and blood lead levels from rehabilitation Bald Eagles and concluded that fecal lead levels were a reasonable predictor of blood lead levels in rehabilitation Bald Eagles. From this regression we created a model to estimate unknown blood lead levels from known fecal lead levels. Our results suggest that feces are a promising method for non-invasively measuring lead exposure when blood is difficult to collect such as in some rehabilitation Bald Eagles or in large-scale field studies involving free-flying raptors or other large birds in the wild. However, the means estimated by the model have considerable variation associated with them, especially at higher lead levels. As a result, there is not a perfect correlation between a given fecal lead

level and a predicted blood level. Our model may be improved with additional paired fecal and blood samples from both rehabilitation and free-flying Bald Eagles.

4) Lastly, we conducted a nest survival study based on nesting, disturbance, and environmental parameters collected during our fecal sampling visits. We did not monitor nests from clutch initiation to fledge. Instead we modeled nest survival from the onset of incubation to when nestlings were 9 weeks old (98 days total), which is shorter than the typical fledging age of 10-12 weeks. Broley (1947) and Anthony et al. (1994) documented that nestling mortality was just small fraction of the total number of causes of nest failure in the Bald Eagles in Florida (2%) and Oregon (3%), suggesting that when food is abundant and fratricide is low, nestling mortality should also be low. Bald Eagle nests in Iowa had high survival rates for the time period we studied. In addition, we found that nest survival was primarily influenced by nest location (survival of nests along the Mississippi River was lower than all other nests), land ownership (survival was higher on public land compared to private land), and proximity to roads (survival was lower near roads). We also estimated that nest initiation dates for Bald Eagle nests found active in the spring ranged from 19 February to 21 March, with a mean estimated nest initiation date of 4 March. Estimated hatch dates for Bald Eagle nests found active in the spring ranged from 26 March to 25 April (mean = 8 April) while estimated fledging dates ranged from 11 June to 11 July (mean = 24 June). Despite the fact that we did not follow nestlings to fledging, this study improves our understanding of nest survival and other vital nesting parameters for Bald Eagles in Iowa. Our short-term nest monitoring suggested that our sampling visits had little to no short term-impact on the behavior of incubating adults and caused no known cases of nest abandonment. We further

infer that our non-invasive sampling method in which we spent <30 minutes within 100 m of the nest had a minimal impact on the nest survival of this species.

LITERATURE CITED

Anthony, R.G., R. W. Frenzel, F.B. Isaacs, and M. G. Garrett. 1994. Probable causes of nesting failures in Oregon's bald eagle population. Wildlife Society Bulletin 22:576-582.

Berglund, A.M., P.K. Ingvarsson, H. Danielsson, and N.E.I. Nyholm. 2010. Lead exposure and biological effects in pied flycatchers (*Ficedula hypoleuca*) before and after the closure of a lead mine in northern Sweden. Environmental Pollution 158:1368-1375.

Broley, C.L. 1947. Migration and nesting of Florida bald eagles. Wilson Bull. 59:3-20.

Dauwe, T., L. Bervoets, R. Pinxten, R. Blust, and M. Eens. 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. Environmental Pollution 124:429-436.

Ek, K.H., G.M. Morrison, P. Lindberg, and S. Rauch. 2004. Comparative tissue distribution of metals in birds in Sweden using ICP-MS and laser ablation ICP-MS. Archives of Environmental Contamination and Toxicology 47:259-269.

Fitzner, R.E., R.H. Gray, and W.T. Hinds. 1995. Heavy metal concentrations in great blue heron castings in Washington state: a technique for monitoring regional and global trends in environmental contaminants. Bulletin of Environmental Contamination and Toxicology 55:398-403.

Morrissey, C.A., L.I. Bendell-Young, and J.E. Elliot. 2005. Assessing trace-metal exposure to American Dippers in mountain streams of southwestern British Columbia, Canada. Environmental Toxicology and Chemistry 24:836-845.

Tiller, B.L., J.D. Marco, and W.H. Rickard. 2005. Metal concentrations, foraging distances, and fledging success of Great Blue Herons nesting along the Hanford Reach of the Columbia River. Environmental Monitoring and Assessment 104:71-79.