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LONG-TERM BALD EAGLE MONITORING: ASSESSING EMERGING RISKS TO THE GREAT LAKES ECOSYSTEM BY EVALUATING IMPACTS TO TERTIARY PREDATORS

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LONG-TERM BALD EAGLE MONITORING: ASSESSING EMERGING RISKS TO THE GREAT LAKES ECOSYSTEM BY EVALUATING IMPACTS TO TERTIARY PREDATORS

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ABSTRACT

Data from the Michigan Bald Eagle Biomonitoring Project (MBEBP) was used to provide ecological evaluations amidst concerns of emerging contaminants and environmental change. Samples and measurements were collected throughout the state of Michigan from 1998-2012. Nestling breast feathers and biometric measurements were used in analytical and statistical analyses which provided a broad assessment of the Great Lakes ecosystem and its resident bald eagles. Bald eagle nestling feathers were taken from Michigan breeding areas, and used to evaluate spatial and temporal trends of mercury (Hg). Overall, remediation has positively affected the Great Lakes region. However, Michigan's Upper Peninsula and Lake Superior shoreline had areas with consistently elevated Hg levels. Possible mechanisms causing increased Hg in these areas are localized coal consumption in addition to atmospheric inputs from the northwestern United States and Asia. This study also addressed concerns regarding nestlings that were exhibiting unusual behavior, morbidity, and/or direct or indirect mortality (i.e. dead sibling or addled egg present) by determining effects from metals/metalloids of concern. In addition to Hg, lead (Pb), cadmium (Cd) and arsenic (As) were measured in nestling feather samples. These metals, although ubiquitous in the environment, were not the primary cause of nestling bald eagle morbidity and mortality observed in the field. It is likely that the expanding population is either experiencing natural causes of mortality, or other mechanisms are causing stress on individual nestlings such as climate or food availability. Lastly, the study evaluated the bald eagle population's response to environmental change by analyzing productivity and biometric measurements of bald

eagle nestlings' bill depth, culmen length, footpad, and hallux claw. The mechanisms acting upon body size clines are complex. There was a correlation between year and deceasing body measurements in nestling bald eagles; however, it is important to recognize that year encompasses several predictors such as food availability. It is likely that several factors are influencing measurements which create difficulties in modeling effects with statistical significance. However, it is notable that statistical significance was achieved in such a short period of time. The utility of the bald eagle as a sentinel species is apparent with the quantity and quality of spatial and temporal trend data that align with other intensive studies. As the Michigan bald eagle population grows and expands into new habitat, so does the capacity for it to provide new information about the environment. The MBEBP not only provides important information regarding the species, it continues to successfully monitor the health of the Great Lakes ecosystem.

DEDICATION

I would like to dedicate this work to my parents, Liz and Dale Fuentes, and my sister Nyco Herzog. Their unending patience, generosity, and support have been invaluable to me throughout my life, and I would not be here without them. I would like to thank Jonathan Flanigan for his help and encouragement. Also, I could not have done this without the kindness of my friends, Bryan and Russell Pearce.

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PREFACE

This dissertation is comprised of three research chapters that are presented as independent manuscripts prepared for submission for publication. Therefore, some redundancy is necessary among sections (i.e. Introduction, Material and Methods, Results, and Discussion).

CHAPTER ONE

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is a tertiary predator in the Great Lakes ecosystem. This piscivorous sea eagle will also prey upon birds, mammals, and reptiles as well as scavenge carrion and steal from other avian predators (Buehler, 2000). Bald eagle populations are susceptible to contaminant exposure and environmental change, and tissue samples and biometric measurements can provide valuable knowledge regarding the effects to species at lower trophic levels. Annual surveys of bald eagle populations have taken place in the United States since the early 1960s when the species was nearly extirpated from the lower 48 states. Currently, bald eagle productivity is increasing and the population is expanding. In Michigan, the statewide population has been monitored since 1961, and these data provide valuable retrospective and current information about the overall health of the Great Lakes ecosystem.

The Great Lakes Basin is the largest body of freshwater in the world with 5,400 cubic miles or 23,000 square kilometers (km^2) of water. This area represents 90 percent (%) of the United States' and 18% of the worlds freshwater. The basin covers 94,000 square miles (245,000 km^2) with 10,200 miles (17,000 km) of shoreline, including 5,000 tributaries. According to the United States (US) Lake Survey (1952), the watershed has a drainage area of 288,000 square miles (746,000 km^2). The entire basin extends from roughly 41 to 51 degrees north latitude and 75 to 93 degrees west longitude (Fuller et al., 1995). Significant ecological systems include terrestrial and wetland inlands, lake plains, tributaries, coastal marshes and shorelines, and open lakes (Rankin and Crispin, 2006).

This ecosystem supports a significant amount of biological diversity that is the basis for complex food webs.

The basin has approximately 1,000 miles (1600 km) of international border (Fuller et al., 1995). In 1978, the Great Lakes Water Quality Agreement (GLWQA) was established between the US and Canada to maintain the chemical, physical, and biological integrity of the waters of the Great Lakes Basin ecosystem (Freedman and Monson, 1989). Both countries are working to meet the objectives of the GLWQA, and are monitored by the International Joint Commission (IJC) (Rankin and Crispin, 2006). The broad objectives of the GLWQA are to ensure that the waters of the Great Lakes are a source of safe, high-quality drinking water; ensure unrestricted recreational uses; allow for safe human consumption of fish and game species; be free from pollutants and nutrients that are above threat thresholds; support healthy wetland habitats and groundwater; and be uninhabited by invasive species (Freedman and Monson, 1989). In order to monitor environmental conditions, the US and Canada were directed to establish and maintain science-based ecosystem indicators to help measure restoration and recovery efforts.

Bald eagles have been recommended as a suitable indicator of Great Lakes' water and habitat quality by the IJC (IJC, 1991; IJC, 1992). According to Ryder and Edwards (1985), the bald eagle is a Type I indicator organism. Such specialized organisms have narrow environmental tolerances, and are sensitive to certain stressors—both naturally occurring and anthropogenic. In order to qualify as a suitable indicator (i.e. sentinel) species, the organism must satisfy the following criteria: 1) be a strong integrator of the

biological food web at one or more trophic levels; 2) be abundant and widely distributed within the system; and 3) be one of perceived human value such that it may be easily sampled (Edwards and Ryder, 1990). The species is long lived and reproduces slowly which makes it susceptible to chronic effects of contaminants. Information can be deduced about lower trophic levels through the concept of contaminant biomagnification. The criteria that enable the species' eligibility as an indicator for environmental contaminants also allow the bald eagle to help estimate changes within its food web. Such changes include land use alterations, shifts in climate, and the availability of prey, and the quality of those factors. In addition, changes can include and be confounded by density-dependent population effects on the bald eagle population.

Both anthropogenic and natural factors can impact the Great Lakes ecosystem. The human population of the basin continues to grow. Currently, the area supports a population of approximately 38 million people (Rankin and Crispin, 2006). Fifty six billion gallons (212 billion liters) of water are used for municipal, agricultural, and industrial purposes. Corn, soybeans, hay, and cherries are the primary crops of the region. The principle industries are steel and auto manufacturing. There are 250 species of fish that support a $4 billion sports and commercial fishery industry. In addition, the Great Lakes Basin provides passage for commercial shipping, cooling water, and a growing emphasis on recreation and tourism (GLERL, 2013). With the human population increase and changes in land use, negative direct and indirect effects of deforestation, agriculturalization, urbanization, eutrophication, overfishing, and the invasion of exotic species are of concern. The health of the Great Lakes fishery began to decline in the

1950s and the chemical water quality of the basin reached peak contamination in the late 1960s and early 1970s (Fuller et al., 1995). Persistent organic pollutants have been monitored throughout the ecosystem, and while some contaminant concentrations are decreasing over time, emerging chemicals may be cause for concern to both human and wildlife populations.

In addition to changes in use of land and water-based resources, the climate of the Great Lakes Basin has shown an observable change over time. The carbon dioxide present in Earth's atmosphere has increased since the industrial revolution from 280 parts per million (ppm) to 350 ppm. Some studies predict that the concentration will reach twice pre-industrial levels by the middle of the next century (Fuller et al., 1995). Based on General Circulation Models, researchers have estimated that the average temperature will increase by 2-4°C at twice the CO_2 level, and lake levels could decrease from 1.6-6.6 feet (0.5-2 m) (Fuller et al., 1995). Human populations may experience direct and indirect impacts of climate change, particularly water resources and use rates. Wildlife also may be affected through the alteration of life history traits such as migration patterns and lay dates for birds as well as their ability to adapt to change.

In 1999, the Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project (Bowerman et al., 2002). This long term monitoring effort provides information about persistent environmental contaminants including polychlorinated biphenyls (PCB), organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), and mercury (Hg) in addition to population productivity and individual bird biometrics. Blood and feather samples and biometrics are

taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of relevant measures. Long-term monitoring has allowed for the determination that bald eagle productivity is increasing spatially and temporally in congruence with the decline of PCB and DDT values below lowest observed adverse effect levels (LOAELS). However, emergent issues have become of concern such as heavy metal bioavailability and contamination in the aquatic ecosystem, and environmental and anthropogenic effects on the increasing bald eagle population. Retrospective analysis of archived samples and data can assist in management strategies of both the aquatic ecosystem and the bald eagle population itself.

Concerns regarding the availability and subsequent effects of non-essential metal and metalloids have become more prevalent with the increase of environmental awareness and improved sampling techniques. Exposed organisms can experience a range of lethal and sub-lethal effects such as reproductive dysfunction, susceptibility to disease or other stresses, changes in behavior, and direct mortality (Scheuhammer, 1987). Biomagnification can also occur, affecting other species among trophic levels. These metals include mercury (Hg), lead (Pb), cadmium (Cd), and the metalloid, arsenic (As). Sources of these metals include industrial products and equipment, wastewater, smelting, mining, pesticides, and recreational uses. The three metals listed above are neither essential nor beneficial to living organisms. Arsenic, however, is abundant and found in trace amounts in all biological tissues. There has been a measurable increase in Hg, Cd, and As in animal tissues; and in acute exposures to Pb in aquatic food chains.

Mercury (Hg) typically enters regions of open water as direct deposition or transported through runoff (Furler et al., 1995; Landis and Keeler, 2002; Rudd, 1995). More specifically, mercury is found in the environment by the following anthropogenic and natural mechanisms: natural deposit in the soil, anthropogenic point sources, and atmospheric deposition (Chan et al., 2003; Driscoll et al., 2005). Once in anaerobic regions, such as those commonly found in wetlands and lake sediments, Hg can be converted to methylmercury (MeHg) (O'Driscoll, 2005; Wiener et al., 2003). Methylmercury is a documented mutagen, teratogen, and carcinogen, and causes embryocidal, cytochemical, and histopathological effects (Eisler, 2007). This compound biomagnifies up the aquatic food chain, with the highest concentrations found in tertiary predators such as the bald eagle. Signs of mercury poisoning in birds include muscular incoordination, falling, slowness, fluffed feathers, calmness, withdrawal, hyporeactivity, hypoactivity, and eyelid drooping. In the case of bald eagles, methylmercury enters the blood stream quickly after ingesting contaminated prey. The kidneys and brain are targeted resulting in mercury toxicosis. Eventually the mercury is either stored in feathers or eliminated (Fournier et al., 2002). This contamination has given cause for concern for the health of humans and piscivorous wildlife.

Lead (Pb) is ubiquitous throughout the environment. The main sources for exposure of bald eagles to Pb is through the ingestion of lead shot and sinkers, or consuming contaminated prey and carcasses. Although nontoxic shot requirements have been established in waterfowl hunting, approximately 3,000 tons (2,722 metric tons) of Pb were expended annually into lakes, marshes, and estuaries by hunters (Eisler, 1988b;

Eisler, 2007; USFWS, 1986; USFWS, 1987). The ban on lead shot in waterfowl hunting took place in 1991, but it is still used in upland hunting, shooting sports, and fishing tackle (NWHC, 2013). Pellets are slowly eroding over time, and are being uncovered by animals and fluctuating water levels. Waterfowl eat the spent pellets, the metal is eroded in the gizzard to a soluble form, and absorbed into the digestive tract. Dead animals or those exhibiting signs of toxicosis are preyed upon by bald eagles resulting in secondary poisoning (Dieter ,1979; Eisler, 1988b; Pattee and Hennes, 1983; Reichel et al., 1984). Lead affects the kidney, bone, central nervous system, and hematopoietic system. It also has neuropsychological, fetotoxic, teratogenic and reproductive affects (Boggess, 1977; DeMichele, 1984; Nriagu, 1978). Bioaccumulation does occur, however, Pb does not biomagnify in food chains. Signs of Pb poisoning are loss of appetite, lethargy, weakness, emaciation, tremors, drooped wings, green liquid feces, and impaired locomotion, balance, and depth perception. Lead inhibits production of blood delta aminolevulinic acid dehydratase (ALAD) which is considered the most sensitive indicator of Pb exposure (EPA, 1979; EPA, 1980; Lumeij, 1985; Schmitt et al., 1984). Often, one pellet is enough to cause mortality; therefore, the increasing bioavailability of Pb is concerning for exposed wildlife populations.

Cadmium (Cd) is a relatively rare metal that has been implicated in deleterious effects to fish and wildlife. Like Hg and Pb, Cd has no biological function. It is used in the electroplating of motor parts, and pigment, plastic stabilizer, and battery production. Wildlife can be exposed to Cd through smelter fumes and dusts, incineration of Cd-bearing materials and fossil fuels, fertilizers, and municipal wastewater and sludge

discharges (Eisler, 1985; Eisler, 2007; Hammons et al., 1978; Hutton, 1983). Animals are protected from most of the negative effects through metallothionein protein binding which renders the element inactive (Eisler, 1985). However, Cd can biomagnify, and effects to bald eagles can be confounded by additional pollutants present in the ecosystem, especially other metals that stimulate metallothionein synthesis. Cadmium affects the liver, kidneys, brain, bone and muscle. It is a teratogen, carcinogen, and can cause sublethal effects such as growth retardation, anemia, renal effects, and testicular damage (Ferm and Layton, 1981; Hammons et al., 1978). Feathers are the target storage depot for Cd. While bald eagles are mostly protected from Cd toxicosis, its increasing prevalence in concert with that of other environmental contaminants point to the need for further assessments of the risk to wildlife and humans.

Arsenic (As) is a metalloid, and is relatively abundant in air, water, soil, and living tissues. A significant amount of arsenicals are released into the environment as a result of agricultural and industrial processes (Eisler, 1988a; Eisler, 2007; Pershagen and Vahter, 1979). Exposure to wildlife can occur through air emission from smelters, coal-fired power plants, and pesticide sprays. Arsenic contamination is also present in water through runoff of mine tailings and smelter wastes. According to Smith et al. (1987) atmospheric deposition has increased over the past 30 years. This metalloid bioconcentrates, but does not magnify. Body burdens are likely due to continuous daily exposure, and As directly destroys blood vessels lining the gut (Nystrom, 1984). Signs of arsenosis are muscular incoordination, debility, slowness, jerkiness, falling hyperactivity, fluffed feathers, drooped eyelid, huddled position, unkempt appearance, loss of righting

reflex, immobility, and seizures (Eisler, 2007). Like Cd, non-lethal doses of As are quickly metabolized and excreted; however, the increase in environmental concentration may pose a risk to sensitive species.

In the presence of increased environmental contaminants, the added stress from changes to the landscape could put pressure on wildlife populations. As stated above, there is evidence that the climate is changing throughout the Great Lakes ecosystem. Alterations in temperature, rainfall, atmospheric CO_2, and water levels not only change the bioavailability of contaminants, the animals themselves may exhibit direct responses to change. More specific to bald eagles, the population has been growing since the 1970s, and new breeding areas are being established in less than desired habitat in close proximity to other bald eagle pairs. This increase in density, decrease in habitat quality, the possible issue of food availability, and a changing climate put pressure on the bird population. Additionally, if the eagle population is responding to environmental stressors, this may be an indication of effects to species at lower trophic levels.

There have been observable changes in bird phenology and life history characteristics. Documented changes have occurred in range distributions, migration patterns and timing, and breeding (Dunn, 2004; Fiedler, 2003; Jonzen et al., 2006; Parmesan, 2006). Recent studies suggest that species of birds are also demonstrating smaller body sizes in response to environmental change (Gardner et al., 2011; Millien et al., 2006; Sheridan and Bickford, 2011; Yom-Tov, 2006). One accepted mechanism to explain this response is phenotypic plasticity (Adger et al., 2007; Parmesan and Yohe, 2003; Teplitsky et al., 2008) in the context of Bergmann's rule. Bergmann's rule is the

inverse correlation between temperature and mean body size of endothermic animals (Teplitsky et al., 2008). The growing and expanding eagle population is being exposed to changes in climate, and may be presenting ecological responses.

The overarching objective of this study is to utilize data from the Michigan Bald Eagle Biomonitoring Project to provide ecological evaluations of the Great Lakes basin. Archived and present nestling feather data were used to evaluate spatial and temporal trends of Hg throughout the state of Michigan. This study will also take a retrospective look at nestlings that were exhibiting unusual behavior, morbidity, and/or direct or indirect mortality (i.e. dead nestling, dead sibling, or addled egg present) to determine any effects from emerging metals/metalloids of concern. In addition, information from data and associated observations may assist as a diagnostic tool in future field seasons. Lastly, the study will evaluate the bald eagle population's response to environmental change by analyzing productivity and biometric data.

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CHAPTER TWO

SPATIAL AND TEMPORAL TRENDS OF MERCURY IN FEATHERS OF NESTLING BALD EAGLES THROUGHOUT MICHIGAN FROM 1986-2012

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is a large bird of prey that is indigenous to North America. This species of sea eagle inhabits areas with large bodies of water with adequate food supply, and prefers super-canopy trees for nesting and roosting. Bald eagles are a top predator in aquatic food chains giving preference to fish, but will also actively hunt birds, mammals, and reptiles as well as scavenge carrion and steal from other predators (Buehler, 2000). During the winter, bald eagles within the Great Lakes region typically do not migrate; however, some birds may fly long distances in order to find food. Bald eagles are considered to be territorial, defending breeding areas consisting of an occupied nest tree and possibly several alternate nests. Eagles reach reproductive age once they are in full adult plumage at 4 to 6 years of age. A breeding pair will attempt to reproduce in one nest per year, and clutch sizes vary from 1-3 eggs (Stalmaster, 1987).

The survival of the species became a topic of concern in the 1960s after a dramatic decrease in the population due to a combination of birds being shot and trapped as varmints, and the exposure and effects of anthropogenic pollutants, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB). Eagle numbers plummeted drastically with only 52 breeding pairs recorded in the state of Michigan in 1961. Bald eagles were placed on the federal Endangered Species List as

Endangered in 1976 throughout its range with the exception of Alaska. This mandated protection afforded the eagle reprieve from shooting, and once DDT and PCB were officially outlawed in 1972 and 1976, respectively, the population began to rebound. Nationwide monitoring efforts were put into place to evaluate population growth with aerial and ground surveys. As of 2012, eagle populations are estimated to be greater than 650 active breeding pairs in Michigan and numbers are still improving (Figure 1.1). The total number of young produced each year has also increased from 34 in 1961 to 676 in 2011 (Figure 1.1). Productivity for each breeding pair was determined by the proportion of total number of young (yng) to the number of occupied breeding areas (occ) each year (Postupalsky, 1974). A productivity value equal to 0.7 yng/occ indicates a stable population and the federal recovery goal associated with a healthy population is 1.0 yng/occ (Sprunt et al., 1973).

Currently, the bald eagle is widely distributed, and has been extensively studied due to its susceptibility to the effects of environmental contaminants such as PCB, DDT, and mercury (Hg) (Bowerman et al., 2002). As a long-lived apex predator, the species is exposed to the effects of biomagnification, and analysis of tissue samples can produce valuable information about organisms positioned lower in the food chain. In addition, bald eagles are territorial nesters that seek out prey items within their breeding area; therefore, samples from nestlings provide a representation of the contaminant levels of the surrounding environment (Bowerman et al., 2002).

Elemental Hg and its compounds have no known metabolic function, but have shown a measurable increase in animal tissues in aquatic food chains. Mercury typically

enters regions of open water by direct deposition or transported through runoff (Hurley et al., 1995; Landis and Keeler, 2002; Rudd, 1995). More specifically, Hg is found in the environment by the following anthropogenic and natural mechanisms: natural deposits in the soil, anthropogenic point sources, and atmospheric deposition (Chan et al 2003; Driscoll et al 2007). Once in anaerobic regions, such as those commonly found in wetlands and lake sediments, Hg can be converted to methylmercury (MeHg). Methylmercury is a highly toxic compound that readily accumulates in organisms and biomagnifies up the food chain to concentrations that exceed those measured in surface water (Chasar et al 2009; Driscoll, 2007; Evers, 2011; Rolfus et al., 2011; Scheuhammer et al., 1987; Wiener et al., 2003). Methylmercury is a documented mutagen, teratogen, and carcinogen, and causes embryocidal, cytochemical, and histopathological effects (Eisler, 2007). As this organic compound biomagnifies, the highest MeHg levels are found in tertiary predators such as the bald eagle. This contamination has given cause for concern for the health of humans and piscivorous wildlife.

Methylmercury contamination of fish, and the associated impairments to water usage, can diminish the recreational, economic, and nutritional benefits of freshwater resources. Eighty percent of fish advisories across the nation are attributed to Hg (USEPA, 2009). Sediment cores from lakes in both hemispheres show that the net Hg deposition has increased threefold since preindustrial times due to an increase in anthropogenic inputs (Bindler et al., 2001; Lamborg et al., 2002; Lindberg et al., 2007). Studies have indicated that Hg methylation has increased by the availability of organic carbon which is stored in organic matter, and actively broken down by microbial activity

(Winfrey and Rudd, 1990). This cycle is especially prevalent in flooded reservoirs and other dynamic wetland habitats.

Wetlands are the primary sites of Hg methylation which expose biota to elevated levels of contamination (Branfireun et al., 2005; Brigham et al., 2009; Chasar et al., 2009; Hurley et al., 1995; Wiener et al., 2006). Michigan contains roughly 15% wetland habitat. The level of MeHg in fish has an inverse relationship with pH and alkalinity (Wiener et al., 1990). The Great Lakes are naturally lower in alkalinity which reduces the area's buffering capacity. According to current climate models, if atmospheric CO_2 continues to trend upward, the lake pH may decline, resulting in conditions that enhance the efficiency of MeHg uptake by fish (Rodgers and Beamish, 1983). Compared to inorganic Hg, MeHg is less volatile, and is more bioavailable for uptake. In addition, Hg methylation has a direct relationship with temperature (Wright and Hamilton, 1982). According to a study conducted by Myers et al. (2009), both average annual minimum and maximum temperatures increased in Michigan over a 37-year period.

Signs of mercury poisoning in birds include muscular incoordination, falling, slowness, fluffed feathers, calmness, withdrawal, hyporeactivity, hypoactivity, and eyelid drooping. Changes in behavior may disrupt and negatively affect foraging and nesting behavior (Jagoe et al., 2002). In wild birds, environmental MeHg exposure may be associated with a higher potential for infection and decreased growth (Harris et al., 2010; Scheuhammer et al., 2007). Hg concentrations in eggs have also been implicated in impaired hatchability and embryonic mortality in a number of bird species (Scheuhammer et al., 2007; Wiener et al., 2003). In the case of bald eagles, MeHg readily

enters the blood stream after ingesting contaminated prey. The kidneys, liver, spleen, muscle, and brain are targeted resulting in Hg toxicosis. Eventually the mercury is either stored in feathers or eliminated (Fournier et al., 2002).

Feathers have been used in previous studies to monitor environmental exposure of birds to mercury (Bowerman et al., 1994; Burger and Gochfeld, 1993; Evers et al., 2005; Monteiro and Furness, 1997; Thompson et al., 1998). As the feathers grow, Hg is stored and accumulated in keratin molecules (Crewther et al., 1965; Thompson et al., 1998). Keratin is not easily degraded; therefore Hg is relatively stable both physically and chemically (Applequist et al., 1984). Studies have shown that nearly all of the Hg found in feathers is MeHg (Thompson and Furness, 1989), and that nestling feather concentrations are indicative of blood levels. These levels provide information about short-term Hg exposure from environmental inputs (Evers et al., 2005).

In 1999, the Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project under the Clean Michigan Initiative. In addition to population productivity and individual bird biometrics, this long term monitoring effort provides information about persistent environmental contaminants including PCBs, organochlorine pesticides such as DDT, and heavy metals such as Hg. Blood and feather samples and biometrics are taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of relevant measures. Long-term monitoring has allowed for the determination that bald eagle productivity is increasing spatially and temporally in congruence with the decline of PCB and DDT values below lowest observed adverse effect levels (LOAELS). However, emergent

issues have become a concern such as Hg bioavailability, the related contamination in the aquatic ecosystem, and whether its presence negatively impacts human and bald eagle populations.

The primary objective of this study was to measure Hg in nestling bald eagle feathers for the purpose of determining spatial and temporal trends throughout the state of Michigan. Temporal trends were evaluated from 1986 to 2012, and the last 10 years of data were compared at four spatial scales. Spatial analyses were conducted at four spatial scales for between 2009 and 2012. Lastly, data were used to and isolate areas of concern.

METHODS

Study Area

The state has been divided into sampling units with a sampling goal of 20% of Michigan's watersheds each year (Figure 1.2). With this design, the entire state was sampled every five years. Feather samples were taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of Hg.

Spatial Analysis

Hg concentrations in nestling feathers were compared at four spatial scales: Statewide; Subpopulation; Great Lakes Watershed; and Individual Watershed (Bowerman et al., 1994; Roe, 2001). The sampling unit for all analyses was the breeding area. Breeding area was defined by an area within an eagle pair's home range that is actively defended, and contains active and inactive nests.

The Statewide spatial scale compared Inland (IN) and Great Lakes (GL) breeding areas. Great Lakes breeding areas were those areas within 8 km (~5 miles) of Great

Lakes shorelines, and along tributaries open to Great Lakes fish. Inland breeding areas were areas located beyond 8 km from shorelines and not along Great Lakes tributaries (Bowerman et al., 1994; Roe, 2001; Bowerman et al., 2003).

The Subpopulation spatial scale subdivided the Statewide spatial scale in order to assign a specific lake affiliation to GL areas, and peninsula to IN areas. The GL subpopulations consisted of breeding areas along Lake Erie (LE), Lake Huron (LH), Lake Michigan (LM), and Lake Superior (LS). The IN subpopulations consisted of Upper Peninsula (IN-UP) and Lower Peninsula (IN-LP) areas.

At the Great Lakes Watershed spatial scale all breeding areas were sorted into nine groupings that were based on Great Lakes Basin drainages. The GL groups were labeled Lake Erie Great Lakes (GL-LE) Lake Huron Great Lakes (GL-LH), Lake Michigan Great Lakes (GL-LM), and Lake Superior Great Lakes (GL-LS). For example, GL-LH areas were all areas that drain into Lake Huron and were within 8 km of the shoreline. The IN groups were Lake Huron Inland Upper Peninsula (IN-UP-LH), Lake Huron Inland Lower Peninsula (IN-LP-LH), Lake Michigan Inland Upper Peninsula (IN-UP-LM), Lake Michigan Inland Lower Peninsula (IN-LP-LM), and Lake Superior Inland (IN-LS). For example, IN-UP-LM were all areas that drain into Lake Michigan, beyond 8 km of the shoreline, and located in the Upper Peninsula.

The Individual Watershed spatial scale was defined by Hydrologic Unit Codes (HUCs) as defined by the United States Geological Survey. These codes identify specific hydrological features such as a river or lake. HUCs were analyzed independently, and then grouped by a larger-scale affiliation: Great Lakes HUCs (GL-HUCs), Inland HUCs

(IN-HUCs), and Mixed IN and GL HUCs (M-HUCs). These are referred to hereafter as "Grouped HUCs."

Temporal analyses were conducted to report changes in overall Hg concentrations over time during four sampling periods: 1986-1992 (T1), 1999-2003 (T2), 2004-2008 (T3), and 2009-2012 (T4). Temporal analyses for Statewide, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales were conducted between T3 and T4.

Field Methods

Aerial Surveys

Michigan Department of Natural Resources (MDNR) pilots and experienced nest observers were contracted to conduct annual aerial surveys. Flights were conducted first in early spring to determine which nests were occupied, and again in late spring to establish which nests were successful. Observers provided the following location information: approximate latitude and longitude of nest tree, nest tree species, reproductive status (e.g., eggs, adult brooding behavior, or chicks). If the nest was successful, observers provided the number of young, stage of nestling development based on size and color, tree condition, and potential nest access from the ground.

Nestling Eagle Capture

Field crews sampled nestlings that were approximately five to nine weeks post-hatch. Lower Peninsula nests were sampled in May, and Upper Peninsula nests were visited in June. Once at the nest, a certified climber ascended the nest tree using spur-climbing techniques, and secured the nestlings in a restraining bag. The bag was lowered

to the ground where it was handled by a trained sample collector. Upon completion of sampling the climber rappelled from the tree.

Sample Collection

Standard handling and sampling procedures were conducted under a United States Geological Survey Bird Banding (USGS) Permit, a United States Fish and Wildlife Service (USFWS) and MDNR Scientific Collecting Permit, and Clemson University Animal Use Protocol. Nestlings were banded using a number nine rivet bird band, and then weighed prior to sample collection. Three to four breast feathers were collected and stored in a coin-sized envelope at ambient temperature until the time of analysis. Biometric measures were taken of the culmen, hallux claw, bill depth, footpad, eighth primary feather to determine the approximate nestling age and gender according to methods published by Bortolotti (1984a; 1984b; and 1984c). Nestlings were placed back into the restraining bag, raised, and released back into the nest. All samples were transferred to Clemson University for analysis via chain-of-custody.

Laboratory Methods

Feather Preparation

Feather samples were washed in a sealed plastic bag using approximately 5% diluted Citranox® and tap water. Next, feathers were rinsed three times with tap water, and three times with reverse-osmosis water. Feathers were then placed into a 2 milliliter (mL) cryogenic vial, covered with folded Chemwipes® that were secured with rubber bands, and stored at -30˚C for 24 hours (h). Once chilled, the feathers were lyophilized for 72 h after which they were placed in a vacuum or stored with desiccant until

digestion. Prior to analysis, 0.05 grams (±0.005g) of each sample were weighed out and placed into 100 mL glass test tubes. Feathers were digested with 10 mL of trace metal grade nitric acid (HNO_3), and each tube was capped using a glass marble. Tubes were placed in a block heater at 80˚C for 30 minutes (min). After, the samples are removed from heat and allowed to reach room temperature for 30 min. Lastly, digested feathers are placed into 250 mL glass jars, diluted to 1:20 (acid to water) using deionized water, sealed with Parafilm®, capped, and stored at room temperature until analysis.

Mercury Analysis

Laboratory analysis was conducted following the United States Environmental Protection Agency Method 245.7. Cold vapor atomic florescence spectroscopy (AFS) was used to analyze and quantify total mercury in each feather sample with an Aurora AI 3200 AFS instrument. Parameters for Hg analysis were as follows: 237.7 nanometer (nm) detector wavelength, 400 mL/min gas flow rate, 60 rpm (reps per min) pump speed, 200˚C atomized temperature, ≥60 seconds (sec), 60 sec, 20 sec, rinse time, update time, and integration time, respectively, 3 replicates, and weight/volume (w/v) $SnCl_2$ in 10% v/v HCl reductant. The AFS detection limit for Hg was 1.0 ng/L.

Mercury concentrations were quantified and verified for quality assurance/quality control using a Hg Reference Standard Solution by Fisher Scientific and prediction curves. An initial standard stock solution of 1000 milligram per kilogram (±1% mg/kg) was used to make five serial dilution standards of 1, 2, 5, 10, and 20 mg/kg. A standard curve was generated from the standards, and quality checks were performed after every

five samples to assure correct instrument standard readings. Optimal recovery rates were set within 85-115% of the original Hg standard curve.

Statistical Methods

Prior to analysis, all Hg concentrations below the AFS detection limit were replaced with a value half the detection limit (0.0005 mg/kg) (Leith et al., 2010). An alpha (α) of 0.05 was used to determine statistical significance. As per convention, all results are reported as geometric means. Statistical analyses were performed using SAS® 9.3. Distributions of Hg concentrations were tested for normality using the Kolmogorov-Smirnov test (PROC UNIVARIATE) and found to be non-normal for both the raw and log-transformed data. Hartley's test also revealed unequal variances between treatments (PROC GLM). Therefore, analyses for overall differences in Hg means between time periods and spatial areas were conducted using rank converted ANOVAs, a nonparametric test equivalent to the Kruskal-Wallis test (PROC RANK; PROC GLM).

When overall differences among the means were detected follow-up analyses were conducted to determine the nature of the temporal and spatial differences. Because examinations of the temporal differences found that simple linear relationships could not satisfactorily describe the changes in contaminant levels through time, post-hoc pair-wise comparisons among the four time periods were conducted using the rank converted Fisher's least significant difference test (LSD) (Wierda, 2009). Pair-wise comparisons among the Statewide, Subpopulation, and Great Lakes Watershed spatial scales were also conducted with the rank converted LSD. Individual watershed analysis involved pair-wise comparisons between 47 watersheds, greatly increasing the overall changes of a

Type I Error. Thus, when comparing the watersheds, rank-converted Tukey's test was used.

RESULTS

Spatial Trends

A total of 226 feather samples were collected from nestling bald eagles throughout the state of Michigan between 2009 and 2012, and were analyzed for Hg. These samples were representative of 183 individual breeding areas. Comparisons of Hg concentrations in nestling feathers were made at the Statewide, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales.

Statewide

Mercury concentrations in feather samples taken from Great Lakes breeding areas were not significantly different from those taken from Inland sites ($F = 3.87^{1, 224}$, $p = 0.0505$). Geometric means for Hg concentrations were 1.41 mg/kg and 1.62 mg/kg for GL and IN, respectively (Table 1.1).

Subpopulation

Mercury concentrations varied significantly among feathers from nestling eagles at the Subpopulation spatial scale ($F = 5.21^{5, 220}$, $p < .0002$). Post-hoc analysis showed statistically similar Hg values in samples from all Inland (IN-LP, IN-UP) sites, and breeding areas along Lakes Superior (LS) and Huron (LH). These spatial scales had the highest geometric mean values of 2.90, 1.82, 1.72 and 1.40 mg/kg for LS, IN-UP, IN-LP, and LH respectively. Great Lakes sites along Lake Michigan (LM) had significantly lower concentrations than IN-UP, but were similar to LH with geometric means of 1.27

mg/kg. In the lower peninsula, Hg concentrations along LM and LE (0.16 mg/kg) were statistically similar and lower than IN-LP (LSD = 1.97, df = 220, $p \leq 0.05$) (Table 1.1).

Great Lakes Watershed

Mercury concentrations in samples at the Great Lakes Watersheds spatial scale varied significantly ($F = 2.65^{10, 215}$, $p = 0.0045$). Inland areas associated with Lake Huron did not differ between peninsulas (IN-UP-LH, IN-LP-LH), and were significantly similar to Lake Huron sites within 8 km of the shoreline (GL-LH). IN-UP-LM and IN-LP-LM did not vary significantly. However, GL-LM sites were significantly lower than IN-UP-LM sites. (LSD = 1.97, df = 215, $p \leq 0.05$). Geometric mean concentrations of Hg were ranked in the following order from lowest to highest: IN-LE (0.04 mg/kg), GL-LE (0.20 mg/kg), GL-LM (1.27 mg/kg), GL-LH (1.40 mg/kg), IN-LP-LM (1.41 mg/kg), IN-UP-LM (1.57 mg/kg), IN-LP-LH (1.93 mg/kg), IN-UP-LH (2.15 mg/kg), IN-UP-LS (2.19 mg/kg), and GL-LS (2.90 mg/kg) (Table 1.1).

Individual Watersheds

Mercury concentrations did vary significantly among 47 Individual Watersheds ($F = 1.61^{46, 178}$, $p = 0.0151$). Further post-hoc analysis (Tukey's) did not show any significant differences. Geometric mean Hg concentrations for Individual Watersheds ranged from 0.04 mg/kg to 4.97 mg/kg (LSD = 5.71, df =178, $p \leq 0.05$).

HUCs were grouped by their affiliation with Great Lakes areas (GL-HUC), Inland areas (IN-HUC), or both (M-HUC). Mercury concentrations varied among Grouped HUCs ($F = 3.21^{2, 222}$, $p = 0.0424$). Concentrations of Hg in Great Lakes HUCs (GL-HUC) were significantly lower than IN-HUC with geometric means of 2.23 and 2.35

mg/kg, respectively. Mixed HUCs were significantly similar to GL-HUC and IN-HUC (1.76 mg/kg).

Temporal Trends

Hg concentrations varied significantly among T1, T2, T3, and T4 ($F = 217.05^{3, 1262}$, $p < 0.0001$). Post-hoc analysis found significant differences between all FOUR time periods (LSD = 1.96, df = 1262, $p \leq 0.05$). The first time period (T1) had the highest Hg concentrations, and then Hg decreased significantly to T2 and T3 with geometric mean Hg concentrations of 7.62 mg/kg, 3.26 mg.kg, and 0.79 mg/kg, respectively. Mercury concentrations increased slightly, but significantly between T3 and T4 with a geometric mean of 1.49 mg/kg. Geometric mean Hg concentrations decreased by approximately 57% between T1 and T2, and approximately 89% between T1 and T3. Hg concentrations increased by 9.5% between T3 and T4 (Figure 1.3).

At the end of the 2012 sampling cycle, the Michigan Bald Eagle Biosentinel Project completed two consecutive five-year cycles (T3 and T4). To assess the unity of continued monitoring, additional post-hoc analyses were conducted to isolate areas of concern as well as areas where remediation and responsible stewardship has proven beneficial to the aquatic ecosystem. Due to the nature of the Project's sampling regime, not all results were significant because of small sample sizes; however, important temporal trends and differences were observed within spatial scales. The Great Lakes Watershed spatial scale was used for analyses because changes in Hg appeared to be localized among individual breeding areas.

There were observable increases in all Great Lake Watershed spatial scales with the exception of GL-LE which had a slight decrease in Hg. There were statistically significant increases in Hg for the following Great Lakes Watershed spatial scales ($p < 0.05$) GL-LS, GL-LH, and IN-LP-LH. Increases were also observed in GL-LM, IN-LP-LM, IN-UP-LM, IN-UP-LH, and IN-UP-LS that were not significant ($p \geq 0.05$). The average decrease in Hg concentration was 0.18 mg/kg between T3 and T4. Eighty-seven of 329 breeding areas that were analyzed had an observable increase in Hg concentrations between T3 and T4. Two of five Great Lakes sites along Lake Erie (GL-LE) had an increase in Hg (0.03 mg/kg and 1.06 mg/kg). Thirteen of 45 GL-LH sites had an increase in Hg (range = 0.02 mg/kg to 2.71 mg/kg). GL-LM had 14 of 44 breeding areas with increasing Hg concentrations (0.02 mg/kg to 2.19 mg/kg). Twelve of 45 GL-LS sites had increasing Hg (0.02 mg/kg to 1.43 mg/kg). Eight of 44 Lower Peninsula Inland sites associated with Lake Huron (IN-LP-LH) were increasing (0.06 mg/kg to 1.14 mg/kg). There was only site in IN-UP-LH, so no comparison was available. Five of 29 and nine of 39 Inland sites were increasing in IN-LP-LM and IN-UP-LM, respectively (0.02 mg/kg to 2.15 mg/kg and 0.62 mg/kg to 7.31 mg/kg). Lastly, seven of 23 IN-UP-LS sites analyzed for Hg were increasing over time (0.28 mg/kg and 6.36 mg/kg).

Additional post-hoc analyses were conducted to observe which specific breeding areas had the five highest Hg concentrations for T3 and T4. Four of 5 breeding areas with the highest Hg concentrations during T3 were located in the Upper Peninsula and associated with Lake Superior (Figure 1.4). These areas were located in Alger (2 sites, GL-LS and IN-UP-LS), Marquette (GL-LS), and Baraga (GL-LS) counties with Hg

concentrations of 10.56 mg/kg, 12.03 mg/kg, 12.60 mg/kg, and 11.03 mg/kg, respectively. The fourth area had a Hg concentration of 10.15 mg/kg, and was located in Missaukee County (IN-LP-LM). All five breeding areas representing the highest Hg concentrations during T4 were located in the Upper Peninsula (Figure 1.4). Four of five breeding areas were located along the Great Lakes shoreline in Marquette, Baraga, and Chippewa counties. It is noteworthy that the nests in Marquette and Baraga counties were sampled at least twice between T3 and T4, and had two of the highest values of Hg in each time period. The Marquette nest had values of 12.60 and 9.52 mg/kg for T3 and T4, respectively. The Baraga County nest had Hg concentrations of 11.03 and 20.97 mg/kg, respectively. The fifth nest was associated with IN-UP-LS in Gogebic County (17.35 mg/kg).

DISCUSSION

This study reports spatial trends in Hg concentrations found in nestling bald eagle feathers during the Michigan Bald Eagle Biomonitoring Project's 2009-2012 sampling cycle, and temporal trends between 1986-1993, 1999-2003, 2004-2008, and 2009-2012. Because Hg biomagnifies, values associated with organisms at high trophic levels provide valuable information about the aquatic ecosystem. Theoretical Hg concentrations can be derived from examining Biomagnification Factors (BMF) which is defined by the known ratio of a contaminant concentration in biota to that in the surrounding water when the biota was exposed to contaminated food (Nowell et al., 2010). As evidenced by the steady increase in the Michigan bald eagle's productivity and expanding population, Hg is currently not negatively affecting the birds themselves. However, some individual

breeding areas may be of concern due to localized increases in Hg concentrations, and/or having consistently high concentrations which may translate into high Hg in nearby fisheries. Sampling efforts should be focused on these areas to further assess any potential risks.

Temporal trends in this study suggest that Hg concentrations have increased slightly within the last five years after experiencing a rapid decline in the proceeding time series. These findings are similar to other studies conducted in the Great Lakes region. Bhavsar et al. (2010) found that that Hg contamination has decreased over the past three decades, but appear to be steady or increasing in fish tissues since 2007. Another study beginning in 1982 also found increasing trends in fish tissues from the mid-1990s to 2006 (Monson, 2009). On the contrary, Levinton and Pochron (2008) and Madsen and Stern (2007) found an overall decline in fish tissues from 1970 to 2004 and 1982 to 2005, respectively. Differences in findings could be attributed to regional differences of atmospheric deposition, and emission and waterborne point sources.

Overall, remediation has positively affected the Great Lakes region. However, with changing climate, land-use practices, and human population, management strategies should be planned cautiously to compensate for these alterations—particularly in Michigan's Upper Peninsula and Lake Superior shoreline. Possible mechanisms causing increased Hg in these areas are localized coal consumption in addition to atmospheric inputs from the northwestern United States. Also, changing climate may be affecting lake pH, water level fluctuations, and temperature (Harris et al., 2010). All of which contribute to the bioavailability of MeHg.

Because bald eagles are known to demethylate Hg, these data may provide a conservative estimate of actual Hg effects to eagles (Scheuhammer et al., 2007). The utility of the bald eagle as a sentinel species is still apparent with the quantity and quality of spatial and temporal trend data that align with other intensive studies. Future studies should focus on localized increased contamination, in addition to the statewide data.

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Table 1. Sample size (n), geometric mean (GM) and range concentration (mg/kg) of mercury in nestling bald eagle feathers collected in Michigan during 2009-2012 time period.

	n	GM	Range[a]
Statewide			
Great Lakes (GL)	138	1.41	ND-20.97
Inland (IN)	88	1.62	ND-17.35
Subpopulation			
Lake Superior (LS)	42	2.90	0.17-20.97
Inland Upper Peninsula (UP)	43	1.82	ND-17.35
Inland Lower Peninsula (LP)	43	1.72	ND-9.20
Lake Huron (LH)	41	1.40	ND-8.55
Lake Michigan (LM)	42	1.27	ND-7.73
Lake Erie (LE)	15	0.16	ND-2.92
Great Lakes Watershed			
Lake Superior Inland (IN-UP-LS)	17	2.19	0.24-17.35
Lake Michigan Inland Upper Peninsula (IN-UP-LM)	24	1.57	ND-8.70
Lake Superior Great Lakes (GL-LS)	42	2.90	0.17-20.97
Lake Huron Inland Upper Peninsula (IN-UP-LH)	1	2.15	.
Lake Huron Inland Lower Peninsula (IN-LP-LH)	27	1.93	ND-9.20
Lake Michigan Great Lakes (GL-LM)	42	1.27	ND-7.73
Lake Erie Great Lakes (GL-LE)	13	0.20	ND-2.67
Lake Erie Inland (IN-LP-LE)	2	0.04	ND-2.92
Lake Huron Great Lakes (GL-LH)	41	1.40	ND-8.55
Lake Michigan Inland Lower Peninsula	16	1.41	ND-5.35

[a.] ND represents a non-detectable Hg concentration which was designated as 0.0005 mg/kg.

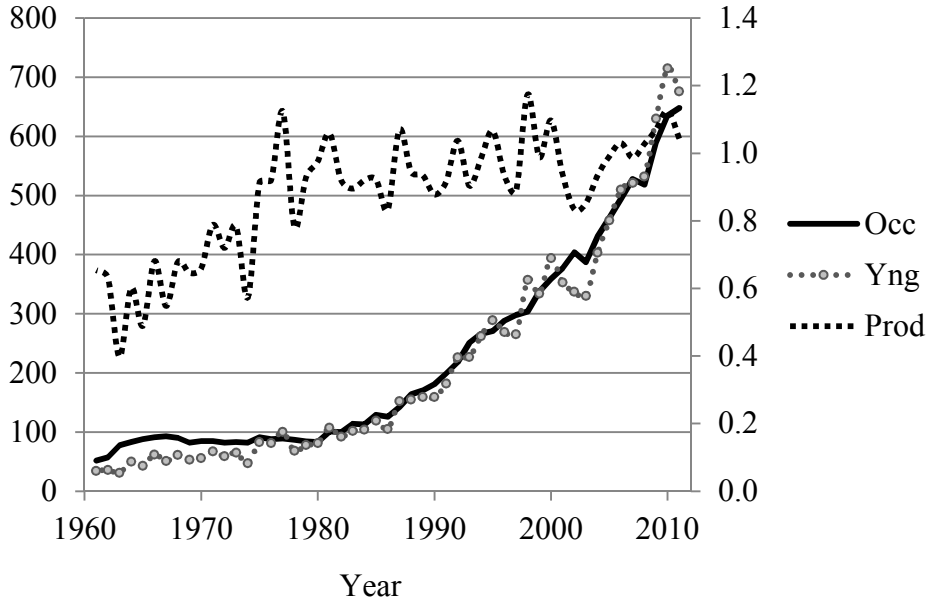

Figure 1.1. Relationship of bald eagle occupied nests (Occ), young produced (Yng), and productivity (Prod) per year from 1961 to 2012.

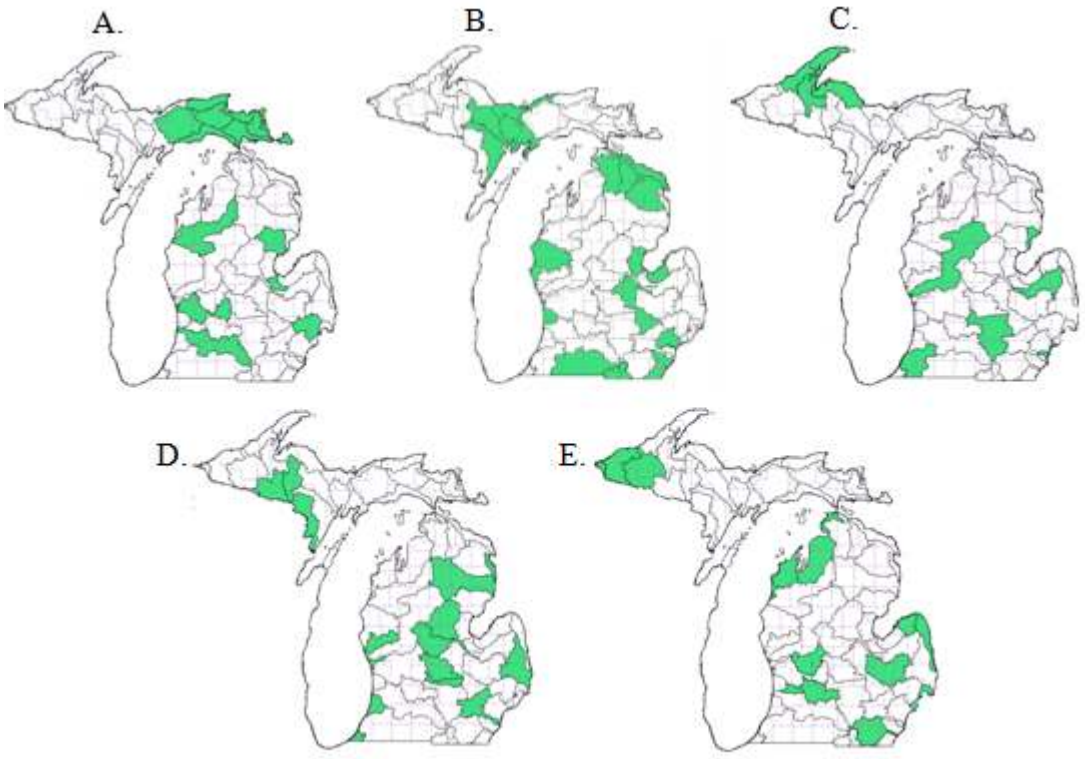

Figure 1.2. Michigan's watershed sampling units (shaded per year). A. 1999, 2004, 2008; B. 2000, 2005, 2009; C. 2001, 2006, 2010; D. 2002, 2007, 2011; and E. 2003, 2008, 2012.

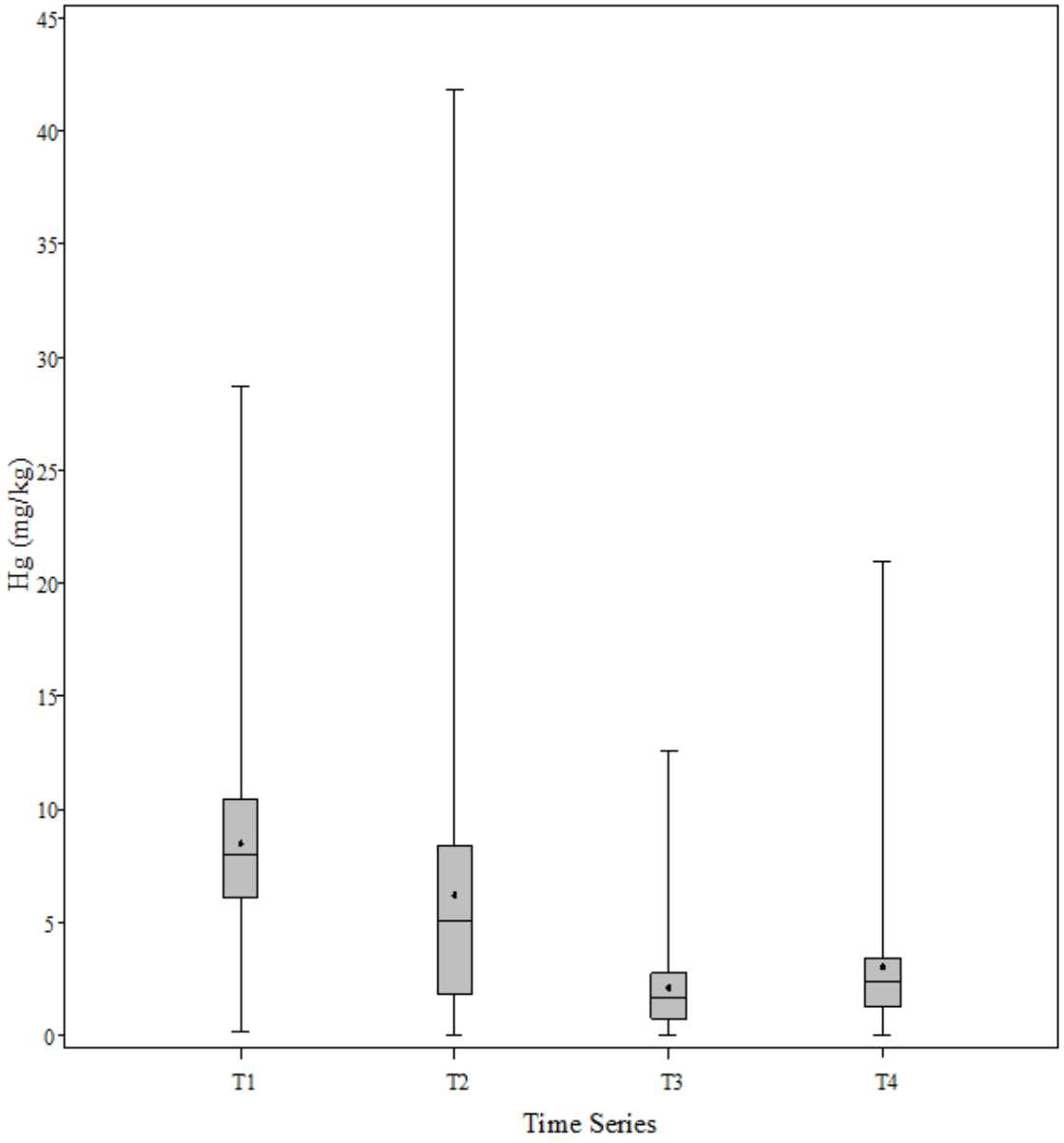

Figure 1.3. Box-and-whisker plot representation of mercury (Hg) concentrations throughout the state of Michigan from 1986-1992 (T1), 1999-2003 (T2), 2004-2008 (T3), and 2009-2012 (T4).

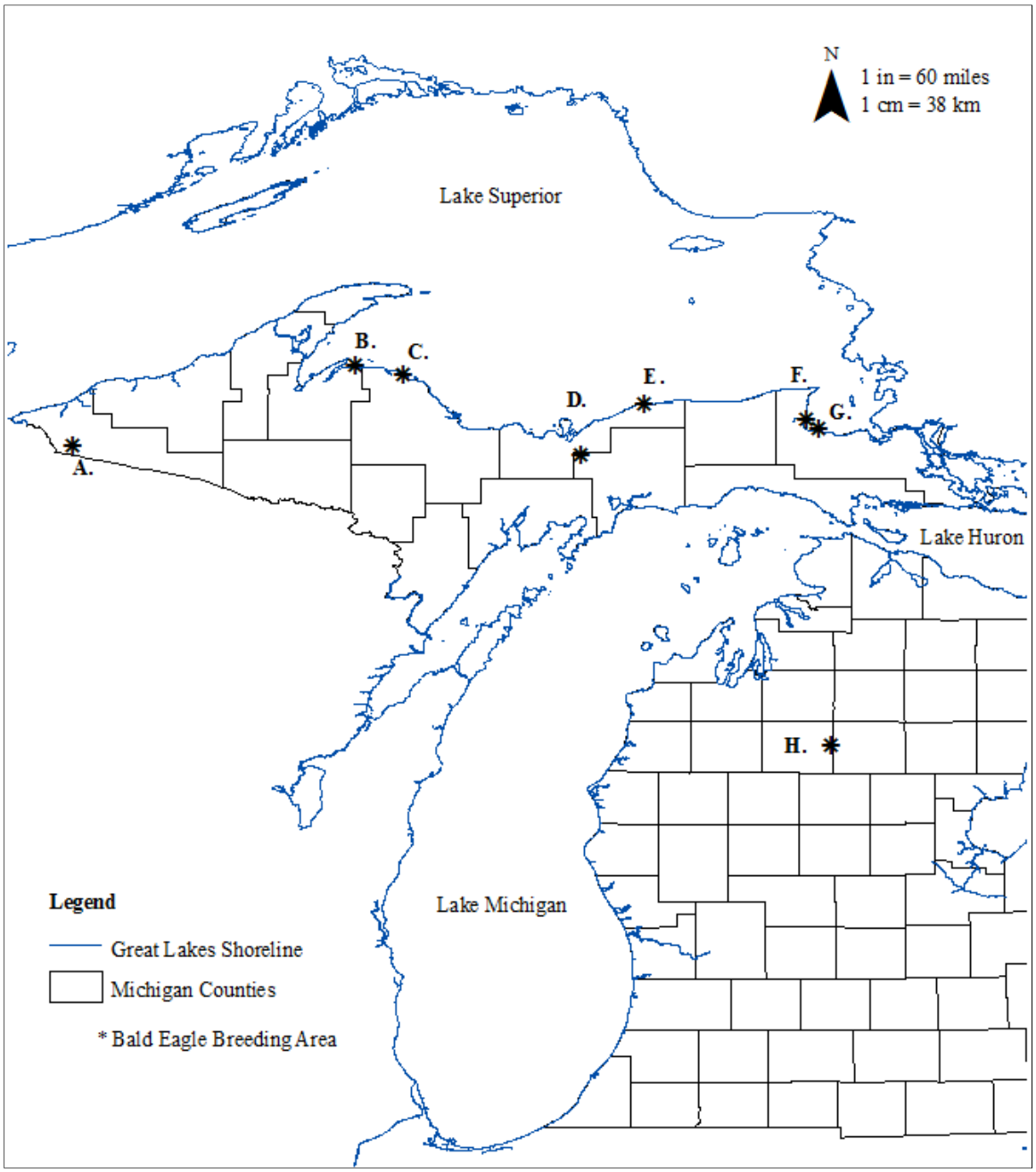

Figure 1.4. Map of Michigan highlighting bald eagle nests with the highest concentrations of mercury between 2004-2008 and 2009-2012 time periods. A. Gogebic County (IN-UP-LS); B. Baraga County (GL-LS); C. Marquette County (GL-LS); D. and E. Alger County (GL-LS); F. and G. Chippewa County (GL-LS); and H. Missaukee County (IN-LP-LM).

CHAPTER THREE

EVALUATING CONCENTRATIONS OF THREE NON-ESSENTIAL METALS AND ONE METALLOID IN NESTLING BALD EAGLES FEATHERS

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is a tertiary predator in the Great Lakes ecosystem. This piscivorous sea eagle will also prey upon birds, mammals, and reptiles as well as scavenge carrion and steal from other avian predators (Buehler, 2000). During the winter, bald eagles within the Great Lakes region typically do not migrate; however, some birds may fly long distances in order to find food. Bald eagles are considered to be territorial, defending breeding areas consisting of an occupied nest tree and possibly several alternate nests. Eagles are a long-lived species that reach reproductive maturity once they are in full adult plumage at 4 to 6 years of age. A breeding pair will attempt to reproduce in one nest per year, and clutch sizes vary from 1-3 eggs. A nest is considered active once eggs are laid, and productive if nestlings hatch after an average incubation period of 35 days (Stalmaster, 1987).

The survival of the species became a topic of concern in the 1960s after a dramatic decrease in the population due a combination of being shot and trapped as varmints, and the exposure and effects of anthropogenic pollutants, dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB). Eagle numbers plummeted drastically with only 52 breeding pairs recorded in the state of Michigan in 1961. Bald eagles were placed on the federal Endangered Species List in 1976 throughout its range with the exception of Alaska. This mandated protection afforded the eagle reprieve from shooting, and once DDT and PCB were officially

outlawed in 1972 and 1976, respectively, the population began to rebound. Nationwide monitoring efforts were put into place to evaluate population growth with aerial and ground surveys. As of 2012, eagle populations are estimated to be greater than 650 active breeding pairs in Michigan and numbers are still improving (Figure 2.1). The total number of young produced each year has also increased from 34 in 1961 to 676 in 2011 (Figure 2.1). Productivity for each breeding pair was determined by the proportion of total number of young (yng) to the number of occupied breeding areas (occ) each year (Postupalsky, 1974). A productivity value equal to 0.7 yng/occ indicates a stable population and the federal recovery goal associated with a healthy population is 1.0 yng/occ (Sprunt et al., 1973). Currently, the bald eagle is widely distributed, and has been extensively studied due to its susceptibility to the effects of environmental contaminants (Bowerman et al., 2002). Monitoring efforts continue, and data provide valuable retrospective and avant-garde information about the overall health of the Great Lakes ecosystem.

Concerns regarding the availability and subsequent effects of non-essential metal and metalloids have become more prevalent with the increase of environmental awareness and improved sampling techniques. In several cases, landowners have inquired about local pollution issues, and how it could affect bald eagles. Communication with wildlife rehabilitators reinforced concerns regarding the prevalence of bald eagle poisonings. The impact of elevated concentrations of contaminants such as metals on the environment can be a significant threat to the stability of the ecosystem (Battaglia et al., 2005). Exposed organisms can experience a range of lethal and sub-lethal effects such as

reproductive dysfunction, susceptibility to disease or other stresses, changes in behavior, and direct mortality (Scheuhammer, 1987). Biomagnification can also occur, affecting other species among trophic levels. Contaminants of interest include mercury (Hg), lead (Pb), cadmium (Cd), and the metalloid, arsenic (As). Heavy metal levels are elevated in ecosystems like the Great Lakes basin because of river influxes, runoff, point-source pollution, and atmospheric transport and deposition (Burger and Gochfeld 2001; Furness and Rainbow, 1990), and bald eagles could be experiencing increased exposure rates. The three metals listed above are neither essential nor beneficial to living organisms. Arsenic, however, is abundant and found in trace amounts in all biological tissues. There has been a measurable increase in Hg, Cd, and As in animal tissues; and in acute exposures to Pb in aquatic food chains. Accumulation of Hg has been documented in aquatic food chains, where sea eagle species (*Haliaeetus spp.*) have shown poor breeding and enhanced mortality (Palma et al., 2005).

Mercury (Hg) typically enters regions of open water as direct deposition or transported through runoff (Hurley et al., 1995; Landis and Keeler, 2002; Rudd, 1995). More specifically, Hg is found in the environment by the following anthropogenic and natural mechanisms: natural deposit in the soil, anthropogenic point sources, and atmospheric deposition (Chan et al., 2003; Driscoll et al., 2005). Once in anaerobic regions, such as those commonly found in wetlands and lake sediments, Hg can be converted to methylmercury (MeHg) (O'Driscoll, 2005; Wiener et al., 2003). Methylmercury is a documented mutagen, teratogen, and carcinogen, and causes embryocidal, cytochemical, and histopathological effects (Eisler, 2007). This compound

biomagnifies up the aquatic food chain. The highest concentrations are found in tertiary predators such as the bald eagle. Signs of mercury poisoning in birds include muscular incoordination, falling, slowness, fluffed feathers, calmness, withdrawal, hyporeactivity, hypoactivity, and eyelid drooping. In the case of bald eagles, methylmercury enters the blood stream quickly after ingesting contaminated prey. The kidneys and brain are targeted resulting in mercury toxicosis. Eventually the mercury is either stored in feathers or eliminated (Fournier et al., 2002). This contamination has given cause for concern for the health of humans and piscivorous wildlife.

Lead is ubiquitous throughout the environment. The main sources for exposure of bald eagles to Pb is through the ingestion of lead shot and sinkers, or consuming contaminated prey and carcasses. Although nontoxic shot requirements have been established in waterfowl hunting, approximately 3,000 tons (2,722 metric tons) of Pb were expended annually into lakes, marshes, and estuaries by hunters (Eisler, 1988b; Eisler, 2007; USFWS, 1986; USFWS, 1987). The ban on lead shot in waterfowl hunting took place in 1991, but it is still used in upland hunting, shooting sports, and fishing tackle (NWHC, 2013). Pellets are slowly eroding over time, and are being uncovered by animals and fluctuating water levels. Waterfowl eat the spent pellets, the metal is eroded in the gizzard to a soluble form, and absorbed into the digestive tract. Dead animals or those exhibiting signs of toxicosis are preyed upon by bald eagles resulting in secondary poisoning (Dieter ,1979; Eisler, 1988b; Pattee and Hennes, 1983; Reichel et al., 1984). Lead affects the kidney, bone, central nervous system, and hematopoietic system. It also has neuropsychological, fetotoxic, teratogenic and reproductive affects (Boggess, 1977;

DeMichele, 1984; Nriagu, 1978). Bioaccumulation does occur, however, Pb does not biomagnify in food chains. Signs of Pb poisoning are loss of appetite, lethargy, weakness, emaciation, tremors, drooped wings, green liquid feces, and impaired locomotion, balance, and depth perception. Lead inhibits production of blood delta aminolevulinic acid dehydratase (ALAD) which is considered the most sensitive indicator of Pb exposure (EPA, 1979; EPA, 1980; Lumeij, 1985; Schmitt et al., 1984). Often, one pellet is enough to cause mortality; therefore, the increasing bioavailability of Pb is concerning for exposed wildlife populations.

Cadmium (Cd) is a relatively rare metal that has been implicated in deleterious effects to fish and wildlife. Like Hg and Pb, Cd has no biological function. It is used in the electroplating of motor parts, and pigment, plastic stabilizer, and battery production. Wildlife can be exposed to Cd through smelter fumes and dusts, incineration of Cd-bearing materials and fossil fuels, fertilizers, and municipal wastewater and sludge discharges (Eisler, 1985; Eisler, 2007; Hammons et al., 1978; Hutton, 1983). Animals are protected from most of the negative effects through metallothionein protein binding which renders the element inactive (Eisler, 1985). However, Cd can biomagnify, and effects to bald eagles can be confounded by additional pollutants present in the ecosystem, especially other metals that stimulate metallothionein synthesis. Cadmium affects the liver, kidneys, brain, bone and muscle. It is a teratogen, carcinogen, and can cause sublethal effects such as growth retardation, anemia, renal effects, and testicular damage (Ferm and Layton, 1981; Hammons et al., 1978). Feathers are the target storage depot for Cd. While bald eagles are mostly protected from Cd toxicosis, the increasing

prevalence in concert with that of other environmental contaminants point to the need for further assessments of the risk to wildlife and humans.

Arsenic (As) is a metalloid, and is relatively abundant in air, water, soil, and living tissues. A significant amount of arsenicals are released into the environment as a result of agricultural and industrial processes (Eisler, 1988a; Eisler, 2007; Pershagen and Vahter, 1979). Exposure to wildlife can occur through air emission from smelters, coal-fired power plants, and pesticide sprays. Arsenic contamination is also present in water through runoff of mine tailings and smelter wastes. According to Smith et al. (1987) atmospheric deposition has increased over the past 30 years. This metalloid bioconcentrates, but does not magnify. Body burdens are likely due to continuous daily exposure, and As directly destroys blood vessels lining the gut (Nystrom, 1984). Signs of arsenosis are muscular incoordination, debility, slowness, jerkiness, falling, hyperactivity, fluffed feathers, drooped eyelid, huddled position, unkempt appearance, loss of righting reflex, immobility, and seizures (Eisler, 2007). Like Cd, non-lethal doses of As are quickly metabolized and excreted; however, the increase in environmental concentration may pose a risk to sensitive species.

Furness et al. (1986) and Burger (1993) consider breast feathers to be the best indicator of whole body burdens of metals. Growing feathers have a blood supply, and provide a storage depot for contaminants (Braune, 1987; Goede and De Bruin, 1984; Goede, 1985; Lewis and Furness, 1991; Monteiro and Furness, 1995). As the feather matures, the vascular connection atrophies, and the feather remains as a non-invasive indicator of metal levels in the blood (Braune and Gaskin, 1987).

In 1999, the Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project (MBEBP) (Bowerman et al., 2002). This long term monitoring effort provides information about persistent environmental contaminants including polychlorinated biphenyls (PCB), organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), and mercury (Hg) in addition to population productivity and individual bird biometrics. Blood and breast feather samples and biometrics are taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of relevant measures. Long-term monitoring has allowed for the determination that bald eagle productivity is increasing spatially and temporally in congruence with the decline of PCB and DDT values below lowest observed adverse effect levels (LOAELS). However, emergent issues have become of concern such as heavy metal bioavailability and contamination in the aquatic ecosystem, and environmental and anthropogenic effects on the increasing bald eagle population. Retrospective analysis of archived samples and data can assist in management strategies of both the aquatic ecosystem and the bald eagle population itself, and be used in predicting future environmental change (Moreno, 2003).

The objectives of this study were to address concerns of heavy metal contamination by evaluating Michigan bald eagle breeding areas that had nestlings exhibiting morbidity and/or mortality from 1999 to 2012. Those nestlings of interest were compared to a control group of healthy ones. Results will assist with assessing the health of the growing eagle population in addition to refining future sampling and laboratory analyses of the Michigan Bald Eagle Biosentinel Project.

METHODS

Study Area

The state has been divided into sampling units with a sampling goal of 20% of Michigan's watersheds each year (Figure 2.2). With this design, the entire state was sampled every five years. Of those samples, feather samples from nestling bald eagles that were exhibiting morbidity, were deceased, or had a deceased sibling or addled egg present were chosen for analyses.

Field Methods

Aerial Surveys

Michigan Department of Natural Resources (MDNR) pilots and experienced nest observers were contracted to conduct annual aerial surveys. Flights were conducted first in early spring to determine which nests were occupied, and again in late spring to establish which nests were successful. Observers provided the following location information: approximate latitude and longitude of nest tree, nest tree species, reproductive status (e.g., eggs, adult brooding behavior, or chicks). If the nest was successful, observers provided the number of young, stage of nestling development based on size and color, tree condition, and potential nest access from the ground.

Nestling Eagle Capture

Field crews sampled nestlings that were approximately five to nine weeks post-hatch. Lower Peninsula nests were sampled in May, and Upper Peninsula nests were visited in June. Once at the nest, a certified climber ascended the nest tree using spur-climbing techniques, and secured the nestlings in a restraining bag. The bag was lowered

to ground where it was handled by a trained sample collector. Upon completion of sampling the climber rappelled from the tree.

Sample Collection

Standard handling and sampling procedures were conducted under a United States Geological Survey Bird Banding (USGS) Permit, a United States Fish and Wildlife Service (USFWS) and MDNR Scientific Collecting Permit, and Clemson University Animal Use Protocol. Nestlings were banded using a number nine rivet bird band, and then weighed prior to sample collection. Three to four breast feathers were collected and stored in a coin-sized envelope at ambient temperature until the time of analysis. Nestlings were placed back into the restraining bag, raised, and released back into the nest. All samples were transferred to Clemson University for analysis via chain-of-custody.

Laboratory Methods

Field data sheets associated with approximately 1300 feather samples from the Michigan Bald Eagle Biosentinel Project 1999-2012 field seasons were collated, and those feather samples that fit the criteria for this study were prepared and analyzed for constituents of interest. A total of 62 of the original 1300 nestling bald eagle feather samples were taken from birds that exhibited signs of morbidity or had a deceased sibling/addled egg in the nest (henceforth referred to as "Morbidity Group"). Morbidity was assigned to nestlings that had unusual behavior, excessive parasites, signs of illness (e.g. respiratory infection) or emaciation, injuries, low weight, and lethargy. In addition, samples were run when a landowner, bystander, or crew member suspected local heavy

contamination. Ten feather samples from nestlings that were considered healthy were randomly selected from existing archives and designated as a Control Group for comparison.

Feather Preparation

Feather samples were washed in a sealed plastic bag using approximately 5% diluted Citranox® and tap water. Next, feathers were rinsed three times with tap water, and three times with reverse-osmosis water. Feathers were then placed into a 2 milliliter (mL) cryogenic vial, covered with folded Chemwipes® that were secured with rubber bands, and stored at -30˚C for 24 hours (h). Once chilled, the feathers were lyophilized for 72 h after which they were placed in a vacuum or stored with desiccant until digestion. Prior to analysis, 0.05 grams (±0.005g) of each sample were weighed out and placed into 100 mL glass test tubes. Feathers were digested with 10 mL of trace metal grade nitric acid (HNO_3), and each tube was capped using a glass marble. Tubes were placed in a block heater at 80˚C for 30 minutes (min). After, the samples are removed from heat and allowed to reach room temperature for 30 min. Lastly, digested feathers are placed into 250 mL glass jars, diluted to 1:20 (acid to water) using deionized water, sealed with Parafilm®, capped, and stored at room temperature until analysis.

Mercury Analysis

Laboratory analysis was conducted following the United States Environmental Protection Agency Method 245.7. Cold vapor atomic florescence spectroscopy (AFS) was used to analyze and quantify total mercury in each feather sample with an Aurora AI 3200 AFS instrument. The AFS detection limit for Hg was 1.0 ng/L. Mercury

concentrations were quantified and verified for quality assurance/quality control using a Hg Reference Standard Solution by Fisher Scientific and prediction curves.

Lead, Cadmium, and Arsenic Analysis

Laboratory analysis was conducted following the United States Environmental Protection Agency Method 200.8 and 6020A. A Thermo Scientific XSeries 2 inductively coupled plasma mass spectrometer (ICP-MS) was used to analyze and quantify metals and metalloids in each feather sample. The ICP-MS detection limit for analysis was 1.0 ng/L. Metal and metalloid concentrations were quantified and verified for quality assurance/quality control using a Mixed Multi-Element Reference Standard Solution by Fisher Scientific and prediction curves.

Statistical Methods

Summary statistics were performed using SAS® 9.3. All results are reported as geometric means, arithmetic means, and ranges in parts per billion (ppb) to facilitate comparisons. Prior to analysis, all Hg, Pb, Cd, and As concentrations below the AFS and ICP-MS detection limits were replaced with a value half the detection limit (0.0005 ppb) (Leith et al., 2010). An alpha of 0.05 was used to determine statistical significance between. Distributions of contaminant concentrations were tested for normality using the Shapiro-Wilk test (PROC UNIVARIATE) and found to be non-normal for both the raw and log-transformed data. Hartley's test also revealed unequal variances between treatments. Analyses for differences between treatments were conducted using the Wilcoxon Rank Sum Test for non-parametric data (PROC NPAR1WAY/WILCOXON).

RESULTS

There was no change in the proportion of nestlings exhibiting morbidity compared to the overall sample numbers over time. Geometric and arithmetic means, and ranges are summarized in Table 2.1. There was no significant difference between Hg and Cd concentrations in the Control and the Morbidity groups ($p > 0.05$). Lead concentrations of the groups were significantly different (Wilcoxon = 506, df = 1, $p = 0.0079$). Arsenic concentrations were also significantly different (Wilcoxon = 275, df = 1, $p = 0.0310$). Additional observation showed that one sample had significantly elevated levels of both Pb and As concentrations (Figure 2.3).

DISCUSSION

Concentrations of non-essential metals Hg, Pb, and Cd, and the metalloid As were analyzed in nestling bald eagle feathers. Birds were selected for analysis because they were exhibiting signs of morbidity, had a deceased sibling or addled egg in the nest, and by the request of researchers and landowners. Levels of Hg and Cd were not significantly different than controls. It should be noted that Hg and Cd have no biological function, and any measureable amount is an indication of exposure. Lead and As levels in the Morbidity group were significantly higher than the Control Group. There was one nestling with highly elevated Pb and As concentrations (Figure 2.3), and when this outlier was removed, concentrations were still significantly different between the two groups. Although small amounts of As can be found in all biological tissues, high levels of As coupled with Pb may point to a lead arsenate pesticide exposure. This arsenical insecticide was used in agriculture as late as 1988 (EPA, 1986), and it is highly persistent in the soil. Contemporary exposure can occur during urbanization projects in areas

formerly used for orchard crops where soils are disturbed, and contaminants become bioavailable. The nestling was not exhibiting signs of toxicosis; however, it had a deceased sibling. It can be speculated that the sibling ingested the food, and was exposed to acute levels, resulting in mortality. The live nestling also had a full crop, so it may not have digested enough of the contaminated prey to exhibit signs of toxicosis.

All feather samples had metal and metalloid concentrations that were considered below background levels. Although no LOAEL values have been established for Hg in eagles, studies suggest a reproductive LOAEL in feathers of 5,000 ppb, and as high as 15,000 ppb of Hg to achieve adverse effects in some predatory birds (Burger and Gochfeld, 2001; Burger and Gochfeld, 2009; Eisler, 2007; Wiener and Spry, 1996). According to Burger and Gochfeld (2001), adverse effects associated with Pb exposure occur at 4,000 ppb in feathers. Cadmium causes sublethal behavior effects at lower concentrations than Hg and Pb, but feather levels have not been determined (Eisler, 2007). Burger (1993) established conversion factors that suggest feather LOAELs would range from 100 ppb to 2,000 ppb. There have been few studies conducted to evaluate the transfer of As through the food chain, and no information is available regarding feather LOAELs in raptors (Erry et al., 1999).

In can be concluded that heavy metals, although ubiquitous in the environment, are not the primary cause of nestling bald eagle morbidity and mortality observed in the field. It is likely that the expanding population is either experiencing natural causes of mortality, or other mechanisms are causing stress on individual nestlings such as climate or food availability. Instances of morbidity and mortality are not resulting in an overall

population decrease at this time as evidenced by productivity numbers (Figure 2.1). However, it is recommended that, if resources permit, Pb be added to annual sampling analyses of the MBEBP.

LITERATURE CITED

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Table 2.1. Geometric mean (GeoMean), arithmetic mean (Mean), and range concentration (ppb) of mercury (Hg), lead (Pb), cadmium (Cd), and arsenic (As) in Morbidity Group and Control Group nestling bald eagle feathers in Michigan from 1999-2012.

Hg (ppb)		
	Morbidity Group	Control Group
GeoMean	0.59	0.02
Mean	0.85	0.50
Range[a]	ND-5.80	ND-1.72
Pb (ppb)[b]		
	Morbidity Group	Control Group
GeoMean	0.10	0.95
Mean	1.12	0.99
Range[a]	ND-30.91	0.56-1.53
Cd (ppb)		
	Morbidity Group	Control Group
GeoMean	0.03	0.03
Mean	0.04	0.04
Range[a]	0.003-0.24	0.02-0.14
As (ppb)[b]		
	Morbidity Group	Control Group
GeoMean	0.002	ND
Mean	0.72	ND
Range[a]	ND-44.28	ND

[a.] ND represents a non-detectable Hg concentration which was designated as 0.0005 ppb

[b.] Represents an element with concentrations that are significant at alpha = 0.05 between Morbidity and Control Groups

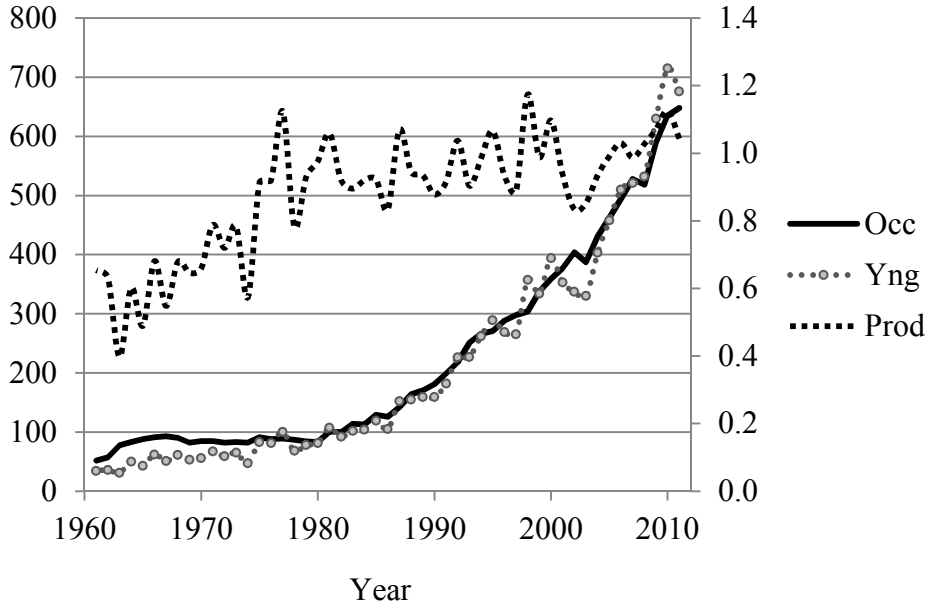

Figure 2.1. Relationship of bald eagle occupied nests (Occ), young produced (Yng), and productivity (Prod) per year from 1961 to 2012.

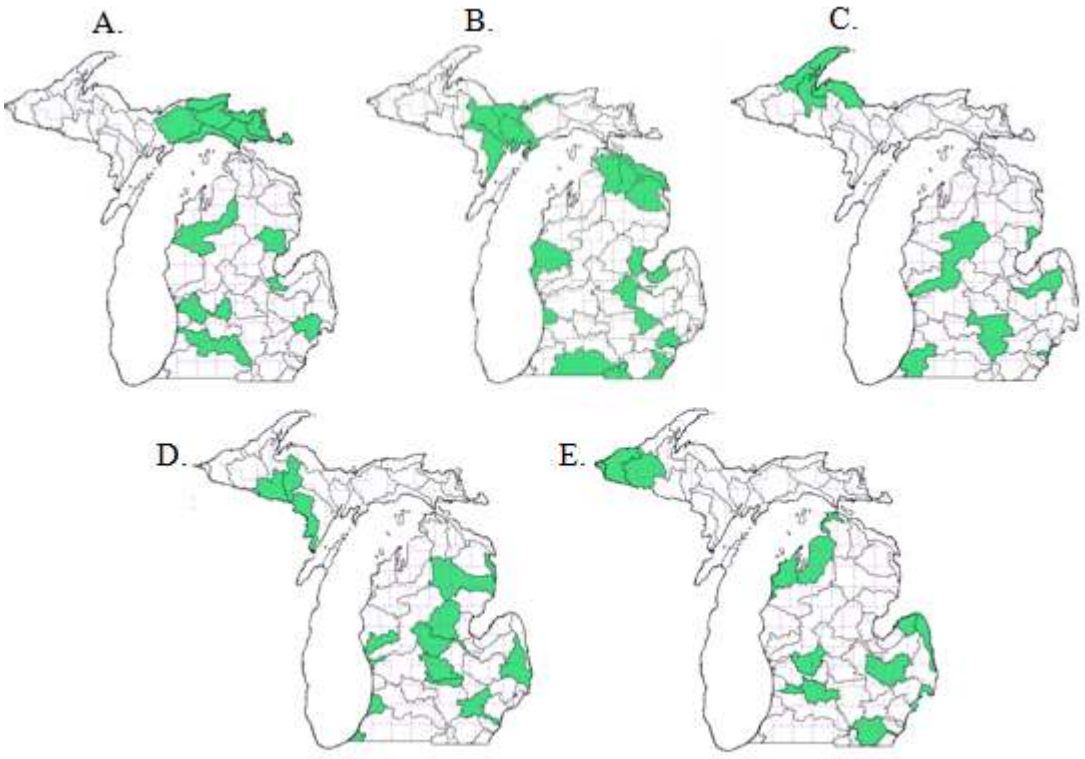

Figure 2.2. Michigan's watershed sampling units (shaded per year). A. 1999, 2004, 2008; B. 2000, 2005, 2009; C. 2001, 2006, 2010; D. 2002, 2007, 2011; and E. 2003, 2008, 2012.

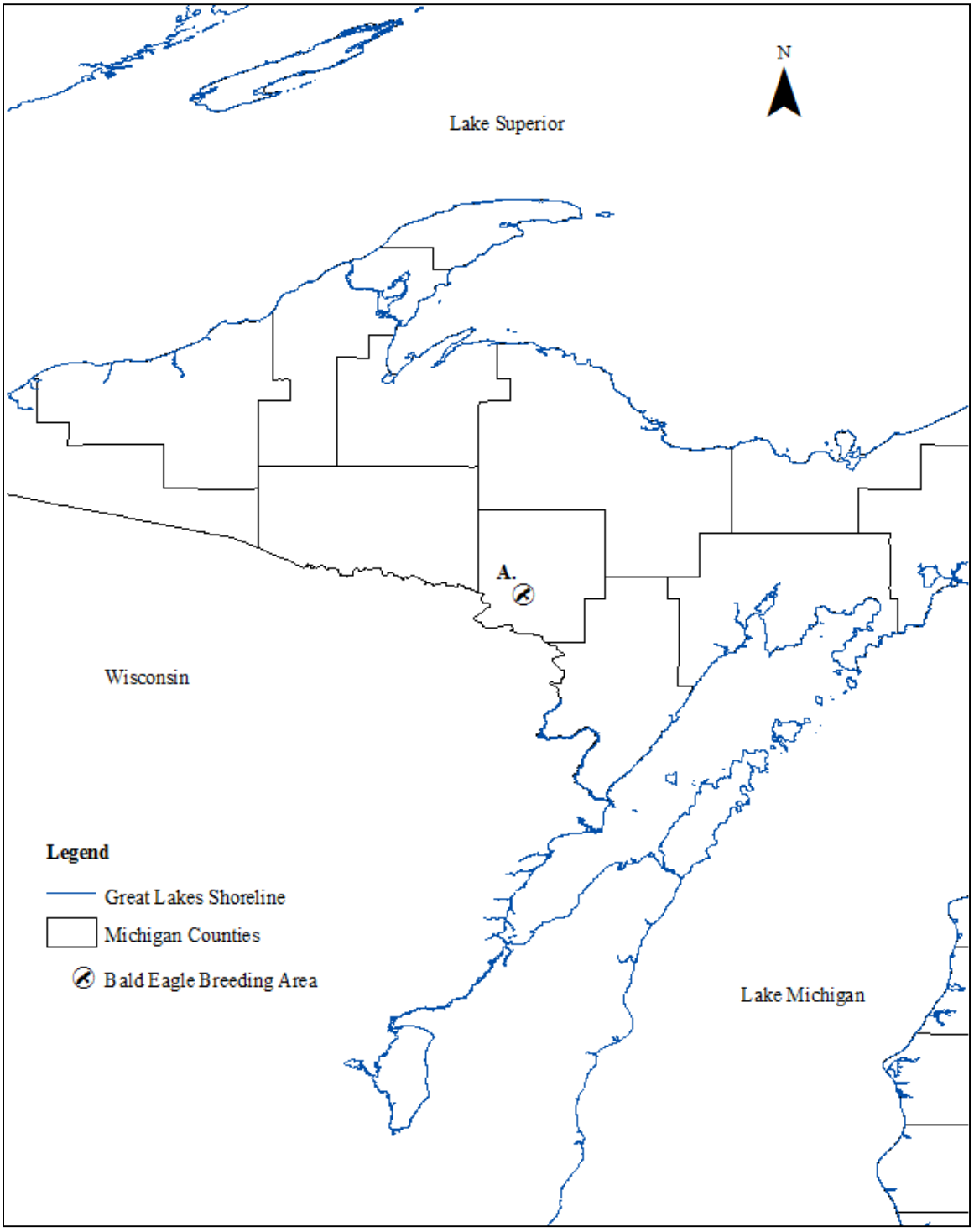

Figure 2.3. Map of Michigan showing eagle nest with possible lead aresenate pesticide poisoning (A) located in Dickinson County.

CHAPTER FOUR

DECREASING BODY SIZE OF NESTLING BALD EAGLES THROUGHOUT MICHIGAN FROM 1998-2012

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is a large bird of prey that is indigenous to North America. This species of sea eagle inhabits areas with large bodies of water with adequate food supply, and prefers super-canopy trees for nesting and roosting. Bald eagles are a top predator in aquatic food chains. This species prefers fish, but will also actively hunt birds, mammals, and reptiles as well as scavenge carrion and steal from other predators (Buehler, 2000). During the winter, bald eagles within the Great Lakes region typically do not migrate; however, some birds may fly long distances in order to find food. Males are smaller than females, and bird populations demonstrate latitudinal clines where southern birds are generally smaller than those to the north. Bald eagles are considered to be territorial, defending breeding areas consisting of an occupied nest tree and possibly several alternate nests. Eagles reach reproductive maturity once they are in full adult plumage at 4 to 6 years of age. A breeding pair will attempt to reproduce in one nest per year, and clutch sizes vary from 1-3 eggs. A nest is considered active once eggs are laid, and productive if nestlings hatch after an average incubation period of 35 days (Stalmaster, 1987).

The survival of the species became a topic of concern in the 1960s after a dramatic decrease in the population due a combination of being shot and trapped as varmints, and the exposure and effects of anthropogenic pollutants,

dichlorodiphenyltrichloroethane (DDT) and polychlorinated biphenyls (PCB). Eagle numbers plummeted drastically with only 52 breeding pairs recorded in the state of Michigan in 1961. Bald eagles were placed on the federal Endangered Species List in 1976 throughout its range with the exception of Alaska. This mandated protection afforded the eagle reprieve from shooting, and once DDT and PCB were officially banned in 1972 and 1976, respectively, the population began to rebound. Nationwide monitoring efforts were put into place to evaluate population growth with aerial and ground surveys. As of 2012, eagle populations are estimated to be greater than 650 active breeding pairs in Michigan and numbers are still improving (Figure 3.1). The total number of young produced each year has also increased from 34 in 1961 to 676 in 2011 (Figure 3.1). Productivity for each breeding pair was determined by the proportion of total number of young (yng) to the number of occupied breeding areas (occ) each year (Postupalsky, 1974). A productivity value equal to 0.7 yng/occ indicates a stable population and the federal recovery goal associated with a healthy population is 1.0 yng/occ (Sprunt et al., 1973). Currently, the bald eagle is widely distributed, and has been extensively studied due to its susceptibility to the effects of environmental contaminants (Bowerman et al., 2002). Along with tissues samples, biometric measurements are typically taken to calculate age and determine sex (Bortolotti 1984a; Bortolotti 1984b; Bortolotti 1984c; Helander, 1981). These measurements could prove useful in evaluating changes in the environment.

There have been observable changes in bird phenology and life history characteristics. Documented changes have occurred in range distributions, migration

patterns and timing, and breeding (Dunn, 2004; Fiedler, 2003; Jonzen et al., 2006; Parmesan, 2006). Recent studies suggest that species of birds are also demonstrating smaller body sizes in response to environmental change (Gardner et al., 2011; Millien et al., 2006; Schmidt and Jensen, 2005; Sheridan and Bickford, 2011; Yom-Tov, 2001; Yom-Tov and Yom-Tov, 2006; Yom-Tov et al., 2006). One accepted mechanism to explain this response is phenotypic plasticity (Adger et al., 2007; Parmesan and Yohe, 2003; Teplitsky et al., 2008) in the context of Bergmann's rule. Bergmann's rule is the inverse correlation between environmental temperature and mean body size of endothermic animals (Bergmann, 1847; Teplitsky et al., 2008). In addition, habitat quality and food availability has been decreasing due to a growing eagle population expanding into areas of anthropogenic habitat fragmentation and degradation (Groombridge, 1992). The growing and expanding eagle population is being exposed to changes in climate and habitat, and may be presenting ecological responses.

Body size affects thermoregulation and energetics of terrestrial endothermic vertebrates. Changing body size has implications for resilience; therefore, it is important to discern sensitive species' response to environmental change (Gardner et al., 2009). As mentioned above, plasticity is a likely cause for biometric changes in shorter time frames, and this mechanism can eventually lead to adaptation. However, according to DeWitt et al. (1998), there are limits to the degree of change that can be achieved in the context of rapid environmental change. This change is apparent in the Great Lakes basin which supports a large eagle population.

Both anthropogenic and natural factors can impact the Great Lakes ecosystem. The human population of the basin continues to grow. Currently, the area supports a population of approximately 38 million people (Rankin and Crispin, 2006). Fifty six billion gallons (212 billion liters) of water are used for municipal, agricultural, and industrial purposes. Corn, soybeans, hay, and cherries are the primary crops of the region. The principle industries are steel and auto manufacturing. There are 250 species of fish that support a $4 billion sports and commercial fishery industry. In addition, the Great Lakes Basin provides passage for commercial shipping, cooling water, and a growing emphasis on recreation and tourism (GLERL, 2013). With the human population increase and changes in land use, negative direct and indirect effects of deforestation, agriculturalization, urbanization, eutrophication, overfishing, and the invasion of exotic species are of concern. The health of the Great Lakes fishery began to decline in the 1950s and the chemical water quality of the basin reached peak contamination in the late 1960s and early 1970s (Fuller et al., 1995). Persistent organic pollutants have been monitored throughout the ecosystem, and while some contaminant concentrations are decreasing over time, emerging chemicals may be cause for concern to both human and wildlife populations.

In addition to changes in use of land and water-based resources, the climate of the Great Lakes Basin has shown an observable change over time. The carbon dioxide present in Earth's atmosphere has increased since the industrial revolution from 280 parts per million (ppm) to 350 ppm. Some studies predict that the concentration will reach twice pre-industrial levels by the middle of the next century (Fuller et al., 1995). Based

on General Circulation Models, researchers have estimated that the average temperature will increase by 2-4°C at twice the CO_2 level, and lake levels could decrease from 1.6-6.6 feet (0.5-2 m) (Fuller et al., 1995). Human populations may experience direct and indirect impacts of climate change, particularly water resources and use rates. Wildlife also may be affected through the alteration of life history traits such as migration patterns and lay dates as well as their ability to adapt to change. Monitoring apex predators such as the bald eagle may provide information about species responses to change at lower trophic levels.

In 1999, the Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project (MBEBP) under the Clean Michigan Initiative. In addition to population productivity and individual bird biometrics, this long term monitoring effort provides information about persistent environmental contaminants including PCBs, organochlorine pesticides such as DDT, and heavy metals such as Hg. Blood and feather samples and biometrics are taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of relevant measures. Long-term monitoring has allowed for the determination that bald eagle productivity is increasing spatially and temporally in congruence with the decline of PCB and DDT values below lowest observed adverse effect levels (LOAELS). However, issues such as environmental change, and the resulting effects on the bald eagle population have become a concern.

It is important for long-term monitoring effects to have robust data, and be adaptable to answer questions about emergent issues. The objectives of the MBEBP have

been to discern effects of contaminants to bald eagles, and in addition to tissue samples, a suite of measurements have been taken to determine age and sex of the nestling bald eagles. These measurements could prove useful in ascertaining possible effects of environmental change.

The objectives of this study were 1) to determine if nesting bald eagle biometrics were changing over the sampling period throughout the state of Michigan; 2) discern differences in biometrics between birds nesting close to Great Lakes shorelines versus those inland; 3) determine if there is a latitudinal change in biometrics between the Upper and Lower peninsulas; 4) distinguish the changes in biometrics among separate the watersheds; and 5) evaluate differences between male and female birds over time.

METHODS

Study Area

The state of Michigan has been divided into units with a sampling goal of 20% of Michigan's watersheds each year (Figure 3.2). With this design, the entire state was sampled every five years.

Temporal Analysis

Temporal analyses were conducted to report changes in overall biometrics over time: 1998-2012. Time periods were then divided up by 5 year time periods: T1 (1998-2002); T2 (2003-2007); and T3 (2008-2012).

Spatial Analysis

Nestling biometrics were compared at three spatial scales: Great Lakes Proximity; Peninsula; and Great Lakes Watershed. The sampling unit for all analyses was the

breeding area. Breeding area was defined by an area within an eagle pair's home range that is actively defended, and contains active and inactive nests.

The Great Lakes Proximity spatial scale compared Inland (IN) and Great Lakes (GL) breeding areas. Great Lakes breeding areas were those areas within 8 km (~5 miles) of Great Lakes shorelines, and along tributaries open to Great Lakes fish. Inland breeding areas were areas located beyond 8 km from shorelines and not along Great Lakes tributaries (Bowerman et al., 1994; Bowerman et al., 2003; Roe, 2001). The Peninsula spatial scale divided the state of Michigan into the Upper Peninsula (UP) and Lower Peninsula (LP). The Great Lakes Watershed spatial scale divided all breeding areas into nine groupings that were based on Great Lakes Basin drainages. The GL groups were labeled Lake Erie Great Lakes (GL-LE) Lake Huron Great Lakes (GL-LH), Lake Michigan Great Lakes (GL-LM), and Lake Superior Great Lakes (GL-LS). For example, GL-LH areas were all areas that drain into Lake Huron and were within 8 km of the shoreline. The IN groups were Lake Huron Inland Upper Peninsula (IN-UP-LH), Lake Huron Inland Lower Peninsula (IN-LP-LH), Lake Michigan Inland Upper Peninsula (IN-UP-LM), Lake Michigan Inland Lower Peninsula (IN-LP-LM), and Lake Superior Inland (IN-LS). For example, IN-UP-LM were all areas that drain into Lake Michigan, beyond 8 km of the shoreline, and located in the Upper Peninsula (Figure 3.3).

Field Methods

Aerial Surveys

Michigan Department of Natural Resources (MDNR) pilots and experienced nest observers were contracted to conduct annual aerial surveys. Flights were conducted first

in early spring to determine which nests were occupied, and again in late spring to establish which nests were successful. Observers provided the following location information: approximate latitude and longitude of nest tree, nest tree species, reproductive status (e.g., eggs, adult brooding behavior, or chicks). If the nest was successful, observers provided the number of young, stage of nestling development based on size and color, tree condition, and potential nest access from the ground.

Nestling Eagle Capture

Field crews sampled nestlings that were approximately five to nine weeks post-hatch. Lower Peninsula nests were sampled in May, and Upper Peninsula nests were visited in June. Once at the nest, a certified climber ascended the nest tree using spur-climbing techniques, and secured the nestlings in a restraining bag. The bag was lowered to ground where it was handled by a trained sample collector. Upon completion of sampling the climber rappelled from the tree.

Biometric measurements

Standard handling and sampling procedures were conducted under a United States Geological Survey Bird Banding (USGS) Permit, a United States Fish and Wildlife Service (USFWS) and MDNR Scientific Collecting Permit, and Clemson University Animal Use Protocol. Nestlings were banded using a number nine rivet bird band, and then weighed prior to sample collection. Biometric measures were taken of the culmen, hallux claw, bill depth, footpad, and eighth primary feather to determine the approximate nestling age and gender according to methods published by Bortolotti (1984a; 1984b; and

1984c). Nestlings were placed back into the restraining bag, raised, and released back into the nest.

Statistical Methods

Statistical analyses were performed using SAS® 9.3. An alpha (α) of 0.05 was used to determine statistical significance. Distributions of measurements were tested for normality using the Kolmogorov-Smirnov test (PROC UNIVARIATE) and found to be non-normal for both the raw and log-transformed data. Hartley's test also revealed unequal variances between treatments (PROC GLM). Regression analyses were conducted to evaluate temporal changes of individual measurements with a multiple analysis of variance option to test the null hypothesis of no overall year effect (PROC GLM/MANOVA). Temporal analyses were conducted to report changes in individual biometrics over time during the entire sampling period, and then divided further into three 5-year sampling periods: 1998-2002 (T1), 2003-2007 (T2), and 2008-2012 (T3). Analyses for differences between spatial treatments were conducted using ranked ANOVAs, a nonparametric test equivalent to the Kruskal-Wallis test (PROC GLM).

RESULTS

A total of 1576 individual nestling bald eagles were measured throughout the state of Michigan between 1998 and 2012. Measurements included footpad, bill depth, culmen length, and hallux claw. Spatial treatments were divided into Great Lakes Proximity; Peninsula; and Great Lakes Watershed spatial scales. The 8th primary measurement was not used because the condition can vary based on weathering and nutrition. In addition,

footpad was not used in spatial comparisons because it was exclusively correlated with gender.

Temporal Trends

There was a statistically significant decrease in all individual measurements from 1998 to 2012 ($F = 13.66^{1, 1464}$, $p < 0.0002$). Multiple analysis of variance determined that overall year effect was statistically significant ($p = <0.0001$). Post-hoc analysis found significant differences between all three time periods for hallux claw (LSD = 0.38, df = 1466, $p \leq 0.05$). Culmen length measurements also significantly decreased between time periods (LSD = 0.50, df = 1470, $p < 0.05$). Bill depth had an observable decrease from T1 to T3, but this was only significant between T1 and T2 (LSD = 0.35, df = 1469, $p < 0.05$). This trend was also discernible in the footpad measurement (LSD = 1.12, df = 1572, $p < 0.05$).

Spatial Trends

Great Lakes Proximity

There was no difference between measurements at the Great Lakes Proximity spatial scale ($p > 0.05$). There were observable decreases in both GL and IN measurements (Table 3.1). The measurements associated with IN sites were more precipitous than in the respective measurements in sites associated with the Great Lakes shoreline (GL).

Peninsula

Measurements taken from nestling eagles varied significantly between the Upper (UP) and Lower Peninsulas (LP) ($p < 0.0002$). All measurements had observable

decreases in the UP. Bill depth and culmen length were significantly decreasing (Table 3.1). Bill depth was significantly decreasing in the LP ($F = 141.94^{2, 727}$, $p < 0.0001$), and culmen length and hallux claw measurements were slightly increasing, but insignificantly.

Great Lakes Watershed

Biometric measurements varied significantly at the Great Lakes Watershed spatial scale ($p < 0.0155$). Samples with less than 100 measurements were not included in this analysis (GL-LE, IN-LE) (Yom-Tov and Geffen, 2011). The following slopes and corresponding p-values are available in Table 3.1. Two of four watersheds associated with the Upper Peninsula (IN-UP-LS, GL-LS) possessed all three nestling measurements that were decreasing. All measurements were decreasing at Inland sites associated with LH, LM, and LS. Bill depth decreased at five of eight watersheds.

Other Trends

Gender

Measurements for male and female nestlings were significantly different ($p < 0.0001$). For males, bill depth was decreasing significantly ($F = 33.54^{1,572}$, $p <0.0001$). Culmen length was also decreasing significantly ($F = 18.35^{1.572}$, $p < 0.0001$) as well as hallux claw ($F = 9.11^{1,571}$, $p = 0.0027$) (Table 3.2). Female bill depth ($F = 55.29^{1,896}$, $p < 0.0001$) and culmen lengths ($F = 8.96^{1,897}$, $p = 0.0034$) were decreasing significantly. The decrease was slightly more apparent in males than in females.

DISCUSSION

Overall, there was a decrease in biometrics in nestling bald eagles over the study period. Bill depth appeared to have the most evident change overall. This notion is not surprising since studies have shown that bird biometrics may not change at the same rate over time (Cooch et al., 1991). Males were decreasing in size significantly faster than females. Females are larger than males, and are able to catch larger prey which may give them an advantage if food is a limiting factor in changing environments. Birds nesting inland experienced a faster rate of change over time compared to those nesting along Great Lakes shorelines. This difference is likely due to inland sites being exposed to greater changes in factors such as temperature and food availability. Also, Upper Peninsula birds had a steeper decrease in biometrics compared to those in the Lower Peninsula which may be an indication of a latitudinal effect. Upper Peninsula sites inland and along Lake Superior may be at higher risk because those birds are already showing significant decreases from change over a short period of time, and the species' limitations are unknown.

Other North American species have experienced changes in body size. Salewski et al. (2010) studied 102 species in the Order Passeriformes from 1961 to 2006. This study showed that body size generally decreased, and the suggested cause was a plastic and/or genetic response to temperature. On the other hand, Meiri et al. (2009) evaluated 22 species in Carnivora from 1900 to 1987, and found variation at the population level, but no change overall. Yom-Tov et al. (2008 and 2007) found an increase in the American marten (*Martes americana*), but a decrease in the body size of lynx (*Felis lynx*) populations over a 50 year study period. Both studies cited food availability as the

primary cause for biometric change. Another charismatic species, the polar bear (*Ursus maritimus*), has also experienced a decrease in body size over the past 20 years (Rode et al., 2010). Species in the Order Rodentia have shown increases, decreases and no change in body size over time due to food availability (Hoffmann and Sgro, 2011; Koontz et al., 2001; Pergrams and Lawler, 2009). Lastly, masked shrew (*Sorex cinereus*) populations have also demonstrated food-dependent increases in body size (Yom-Tov and Yom-Tov, 2005). According to the studies, effects can be found at multiple trophic levels which could have larger implications that give cause for concern.

Changes in phenology can have multiple, confounding effects on high risk, taxonomically diverse animal populations (Hughes, 2000; Parmesan and Yohe, 2003; Parmesan, 2006). These effects include changes in the timing of life cycle events, and poleward and altitudinal shifts in species distribution (Gardner et al., 2009). Sublethal effects range from increase in disease and parasite prevalence (Poulin, 2011), inter- and intraspecific competition (Brown and Wilson, 1956; Dayan and Simberloff, 1998), and predation pressure (Gentle and Golser, 2001; Gosler et al., 1995). According to Diamond (1984), and Crooks and Soule (1999), species at higher trophic levels, like the bald eagle, are more vulnerable to the cumulative effects of disturbance to species at lower trophic levels. The bald eagle's slow life history and geographic dependence upon large bodies of water make the species susceptible to population decline (Purvis et al., 2000).

The mechanisms acting upon body size clines are complex. As evidenced in this study, there is correlation between year and body measurements; however, it is important to recognize that year encompasses several predictors (Yom-Tov and Geffon, 2011) such

as food availability. According to studies, weather-driven food availability is an important predictor of body size (Ho et al., 2010; Ohlsson and Smith, 2001; Searcy et al., 2004; White, 2008; Yom-Tov and Geffon, 2011). In addition, climate studies have related changes to global warming. When adequate climate data are unavailable or unreliable, latitude has been used as a proxy (Mayr, 1963). In this study, it is likely that several factors are influencing measurements which create difficulties in modeling effects with statistical significance. However, it is notable that statistical significance was achieved with biological relevance in such a short period of time.

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Table 3.1. Trends of nestling bald eagle biometric measurements throughout the state of Michigan from 1998-2012.

Great Lakes Proximity					
	Slope			Slope	
GL			**IN**		
Bill	-0.10		Bill	-0.17	
Culmen	-0.05		Culmen	-0.18	
Hallux	-0.04		Hallux	-0.10	
Peninsula					
	Slope	p-value[a]		Slope	p-value[a]
LP			**UP**		
Bill	-0.07	<0.0001*	Bill	-0.12	<0.0001*
Culmen	0.04	0.1101	Culmen	-0.08	0.0003*
Hallux	0.02	0.2285	Hallux	-0.03	0.1166
Great Lakes Watershed					
	Slope	p-value[a]		Slope	p-value[a]
GL-LH			**IN-UP-LH**		
Bill	-0.11	0.0036*	Bill	-0.32	0.1694
Culmen	-0.04	0.5192	Culmen	-0.64	0.0599
Hallux	-0.06	0.1857	Hallux	-0.29	0.1748
GL-LM			**IN-UP-LM**		
Bill	-0.06	0.0991	Bill	0.00	0.8976
Culmen	0.03	0.4946	Culmen	0.05	0.3202
Hallux	0.01	0.6931	Hallux	0.11	0.0053*
GL-LS			**IN-UP-LS**		
Bill	-0.18	<0.0001*	Bill	-0.24	<0.0001*
Culmen	-0.18	0.0025*	Culmen	-0.26	<0.0001*
Hallux	-0.11	0.0203*	Hallux	-0.19	0.0002*
IN-LP-LH			**IN-LP-LM**		
Bill	-0.20	<0.0001*	Bill	-0.23	<0.0001*
Culmen	-0.21	<0.0001*	Culmen	-0.22	<0.0001*
Hallux	-0.16	<0.0001*	Hallux	-0.13	<0.0001*

[a.] The "*" symbol represents a value that is significant at alpha = 0.05.

Table 3.2. Differences in biometric measurements between male and female nestling bald eagles throughout Michigan from 1998-2012.

	Gender				
	Slope	p-value[a]		Slope	p-value[a]
Male			**Female**		
Bill	-0.11	<0.0001*	Bill	-0.11	<0.0001*
Culmen	-0.12	<0.0001*	Culmen	-0.07	0.0034*
Hallux	-0.06	0.0027*	Hallux	-0.02	0.1632

[a.] The "*" symbol represents a value that is significant at alpha = 0.05.

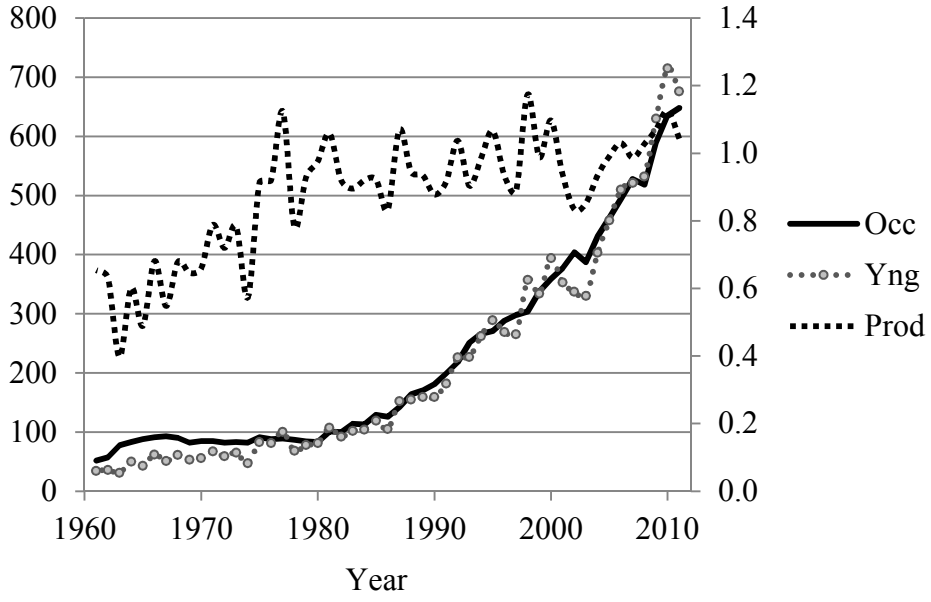

Figure 3.1. Relationship of bald eagle occupied nests (Occ), young produced (Yng), and productivity (Prod) per year from 1961 to 2012.

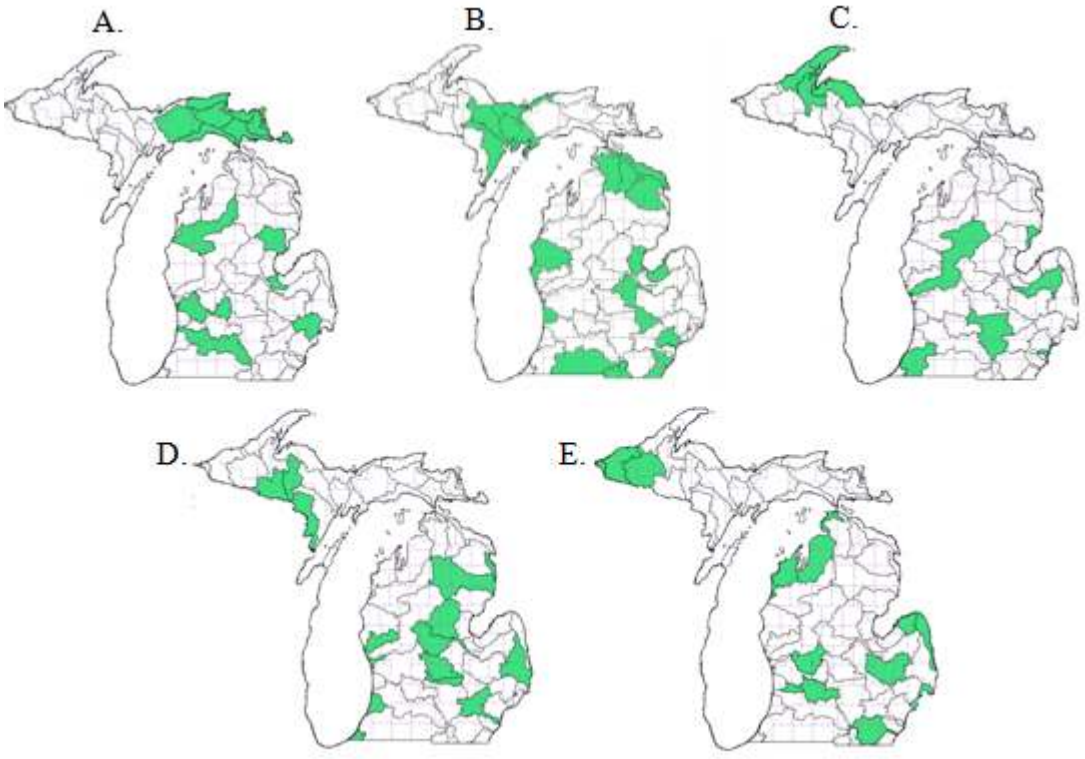

Figure 3.2. Michigan's watershed sampling units (shaded per year). A. 1999, 2004, 2008; B. 2000, 2005, 2009; C. 2001, 2006, 2010; D. 2002, 2007, 2011; and E. 2003, 2008, 2012.

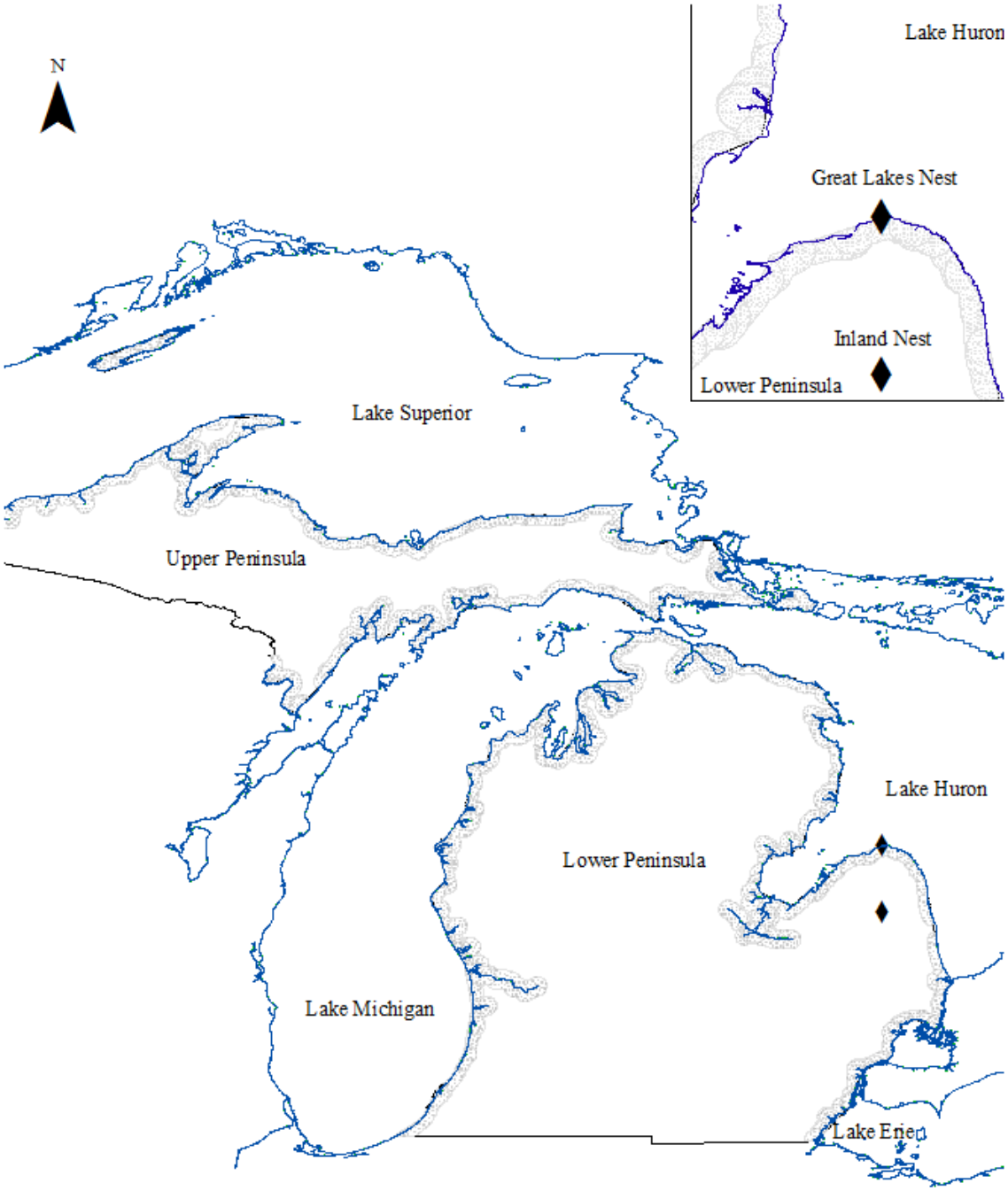

Figure 3.3. Map of Michigan and surrounding Great Lakes highlighting spatial scales: Upper Peninsula (UP); Lower Peninsula (LP); Lake Superior (LS); Lake Michigan (LM); Lake Huron (LH); Lake Erie (LE); and inset: Shaded Great Lakes shoreline (GL); Inland (IN).

CHAPTER FIVE

SUMMARY

The main objective of this study was to utilize data from the Michigan Bald Eagle Biomonitoring Project to provide ecological evaluations. Data were collected throughout the state of Michigan from 1998-2012. Nestling breast feathers and biometric measurements were used in analytical and statistical analyses which provided a broad assessment of the Great Lakes ecosystem and its resident bald eagles.

Archived and present nestling feather data were used to evaluate spatial and temporal trends of Hg throughout the state of Michigan. Overall, remediation has positively affected the Great Lakes region. However, with changing climate, land-use practices, and human population, management strategies should be planned cautiously to compensate for these alterations—particularly in Michigan's Upper Peninsula and Lake Superior shoreline. Possible mechanisms causing increased Hg in these areas are localized coal fired electrical plants in addition to atmospheric inputs from the northwestern United States or Asia. Changing climate may be affecting lake pH, water level fluctuations, and temperature which ultimately contribute to the bioavailability of the highly toxic MeHg.

This study addressed concerns regarding nestlings that were exhibiting unusual behavior, morbidity, and/or direct or indirect mortality (i.e. dead nestling, dead sibling, or addled egg present) by determining effects from metals/metalloids of concern. It can be concluded that heavy metals, although ubiquitous in the environment, are not the primary cause of nestling bald eagle morbidity and mortality observed in the field. It is likely that

the expanding population is either experiencing natural causes of mortality, or other mechanisms are causing stress on individual nestlings such as climate or food availability. Instances of morbidity and mortality are not resulting in an overall population decrease at this time as evidenced by productivity numbers.

Lastly, the study evaluated the bald eagle population's response to environmental change by analyzing productivity and biometric data. The mechanisms acting upon body size clines are complex. As evidenced in this study, there is correlation between year and deceasing body measurements in nestling bald eagles; however, it is important to recognize that year encompasses several predictors such as food availability. It is likely that several factors are influencing measurements which create difficulties in modeling effects with statistical significance. However, it is notable that statistical significance was achieved with biological relevance in such a short period of time.

The utility of the bald eagle as a sentinel species is apparent with the quantity and quality of spatial and temporal trend data that align with other intensive studies. It is recommended that sampling efforts continue as-is, and special consideration should be taken when choosing breeding areas to sample in the Upper Peninsula, particularly along the Lake Superior shoreline. Analytical analyses should include Pb measurements, and researchers should consider running archived samples for Pb levels to obtain spatial and temporal trends throughout the state. Biometric measurements should continue to be taken, and training should emphasize quality assurance and quality control. The Michigan bald eagle population is growing and expanding into new habitat. The MBEBP not only

provides important information regarding the species, it continues to successfully monitor the health of the Great Lakes ecosystem.