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USING BALD EAGLES TO TRACK SPATIAL AND TEMPORAL TRENDS OF CONTAMINANTS IN MICHIGAN'S AQUATIC SYSTEMS

Michael Ray Wierda
Clemson University

USING BALD EAGLES TO TRACK SPATIAL AND TEMPORAL TRENDS OF CONTAMINANTS IN MICHIGAN'S AQUATIC SYSTEMS

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Michael Ray Wierda
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Accepted by:
Dr. William W. Bowerman, Committee Chair
Dr. William C. Bridges
Dr. Joseph D. Lanham
Dr. James G. Sikarskie

ABSTRACT

The bald eagle (*Haliaeetus leucocephalus*) is an extensively researched tertiary predator. Studies have delineated information about its life history and the influences of various stressors on reproduction. Due to the bald eagles position at the top of the food web, it is susceptible to biomagnification of a wide array of xenobiotics. In Michigan the bald eagle population has recovered strongly since the population bottle-neck of the 1960s. In the 1960s when Michigan's eagle population was first being monitored less than 100 nests were occupied yearly (i.e., active breeding pairs existed). Today there are approximately 500 occupied nests each year and over 700 breeding areas in the state.

Because p,p'-dichlorodiphenyltrichloroethylene (p,p'-DDE), PCBs and Hg are often all found in individual nestling eagles and eagle eggs it is hard to establish a causative effect between individual persistent chemical and declined reproduction. Field studies and laboratory work has shown a correlation between p,p'-DDE, PCBs, and Hg concentrations and decreased reproduction success. In the shell gland of birds, DDE inhibits the action of carbonic anhydrase which is necessary to supply the carbonate ions used in shell formation. PCBs have been correlated with dead and deformed embryos of water birds in the upper Great Lakes. Field and laboratory studies have also correlated Hg concentrations with behavioral changes, which may disrupt foraging and nesting.

The Michigan Department of Environmental Quality (MDEQ) implemented a monitoring program using the bald eagle to monitor trends of persistent chemicals under the Clean Michigan Initiative in 1999. These monitored persistent chemicals included PCBs, organochlorine pesticides, and Hg.

The state was divided into major "watershed years" with 20% of Michigan's watersheds being sampled each year. This sampling procedure allowed for the entire state to be sampled and analyzed every five years. During annual banding activities, blood and feather samples from nestling bald eagles were collected within these designated watersheds. Monitoring contaminant trends at various spatial scales allows for comprehensive assessment of the Great Lakes Basin ecosystem health.

The objectives of this research were to evaluate spatial and temporal trends of Hg, PCBs and pesticides in nestling bald eagles of Michigan. For Hg, spatial and temporal trends were determined. For PCBs and pesticides only spatial trends were examined because some data were not available at the time of writing. As data become available further analysis will be conducted, including temporal trends.

In the first study "Using nestling bald eagles to track spatial trends of PCBs and pesticides in aquatic ecosystems of Michigan" we evaluated PCB and pesticide concentrations at three spatial scales. In summary, our study found that concentrations of PCBs and pesticides were significantly higher in Great Lakes areas with Lakes Michigan and Huron having highest concentrations of pesticides and Lake Erie having highest concentrations of PCBs.

In the second study "Using nestling bald eagles to track spatial and temporal trends of mercury in aquatic ecosystems of Michigan." we evaluated Hg concentrations at four spatial scales and three temporal periods. In summary, our study found that Hg concentrations where significantly greater in the Upper Peninsula of Michigan and inland

areas and that while concentrations have decreased from those of the late 1980s they are currently increasing across the state.

Continued monitoring of bald eagle populations is suggested for several reasons. First, nestling blood and feather contaminate levels have been shown to be an appropriate method to monitor ecosystem contaminant levels. Both blood and feather samples can be collected during routine nestling banding activities. Second, both PCB and pesticide concentrations for 37% and 40% of the nestling eagles sampled were above the no observable adverse effect level for bald eagles. Thus, it is possible that once these nestlings reach sexual maturity, they may not be able to reproduce at a level considered necessary to support a healthy population due to elevated concentrations of DDE or PCB. Lastly, with Hg concentrations on the rise, adverse effects including decreased reproduction could occur in bald eagles. The Upper Peninsula of Michigan should be concentrated on because of its characteristics which lead to increased bioavailability of Hg. With the current concentrations of PCBs and pesticides and increasing Hg concentrations this ongoing research may be in a unique position to document the threshold at which detrimental effects from persistent xenobiotics occur in the bald eagle.

DEDICATION

I dedicate this dissertation to my parents, Ronald and Susan Wierda, who always knew I had it in me.

ACKNOWLEDGEMENTS

I thank my friends and family (blood and extended), for all of their support on this long strange trip. You have all kept me happy or grounded in one way or another. I thank my school mates whose like-minded-ness keep me feeling rational or even relaxed on occasion. I thank Norman Ellis and Dr. Wayne Chao for all their help. I thank David Best at the USFWS in East Lansing for his support in this project. I thank Dr. Peter Marko for all his mentoring and unwavering patience. A big thanks to Teryl Grubb for all his mentoring in the field on eagle ecology, tree climbing, and numerous other things.

I thank my committee members; Dr. James Sikarskie, Dr. Drew Lanham, and Dr. William Bridges for all their mentoring. I especially thank Dr. William Bowerman my mentor for the last 5 years and committee chair. I thank you for all the opportunities with which you have presented me, the learning opportunities they have become, and countless hours of mentoring.

Lastly, I thank over and over again Katherine Ferne Leith for all her help and support with life the universe and everything.

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PREFACE

This dissertation was written in journal style and organized into two chapters, each with an introduction, methods, results, and discussion. Each chapter is intended for publication and repetition in some sections (i.e. Introduction, Methods, Results, Discussion, and Literature Cited) may occur. The chapters are preceded by an Introduction and followed by overall Conclusions.

INTRODUCTION

BALD EAGLE STATUS

The bald eagle (*Haliaeetus leucocephalus*) is one of the most studied birds of North America. Hundreds of scientific studies have delineated information about its life history, including the influence of various stressors on reproduction (Bowerman et al. 2002). The bald eagle is a large bird of prey and an opportunistic forager, generally preferring fish over a variety of avian, mammalian, and reptilian prey (Buehler 2000). Bald eagles are associated with aquatic habitats (coastal areas, rivers, lakes, reservoirs, and forested shorelines) of North America. Estimates of territory size vary widely based on nesting density, food supply, and method of measurement (Buehler 2000). Bald eagles typically lay one to three eggs per clutch with a mean clutch size of 1.87 (Stalmaster 1987) and both sexes assist in incubation and rearing of young.

The bald eagle is a tertiary predator of the Great Lakes Basin aquatic food web. Due to its position at the top of the food chain, this species is susceptible to biomagnification of a wide array of xenobiotics; mercury (Hg), methylmercury (MeHg), polychlorinated biphenyls (PCBs), and pesticides including dichloro-diphenyl-trichloroethanes (DDT) and its metabolites (DDTs), and other organochlorines (OCs). The bald eagle has been proposed as a biological indicator of exposure to toxic organochlorines and metal compounds for pisciverous wildlife and as a monitor of the effects of contaminant bioaccumulation and biomagnification in the Great Lakes (International Joint Commission 1994, State of the Lake Ecosystem Conference 1998, 2000). The bald eagle is ideal as a biosentinel for several reasons: it is indigenous to

Michigan, it interacts directly with the environment, it has a quantified niche (i.e., is a piscivor), it is important to humans, and it does not duplicate current indicators.

The bald eagle population in Michigan has recovered strongly since the population bottle-neck of the 1960s. In the 1960s when Michigan's eagle population was first being monitored less than 100 nests were occupied (i.e., active breeding pairs existed). Today there are approximately 500 occupied nests each year (Figure 1) and over 700 breeding areas in the state. Productivity within each area was determined by dividing the total number of young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Productivity have increased throughout Michigan. Productivity of the 1960s was 0.59 compared to the recent (2000-2006) productivity of 0.95. Success was determined by dividing the number of nests producing fledged young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Success (# successful nest/# occupied territories) have also increased. Success rates of the 1960s were 0.41 compared to recent success rates of 0.62. With increases in population size, Productivity, and Success the number of nestling bald eagle produced each year has also increased. In the 1960s < 50 nestling eagles were produce, in recent years (2000-2006) > 400 nestling eagles have been produced each year (Figure 1).

Because p,p'-dichlorodiphenyltrichloroethylene (p,p'-DDE), PCBs and Hg are often all found in individual nestling eagles and eagle eggs it is hard to establish a causative effect for an individual persistent chemical. However past data and laboratory studies suggest that DDE, PCBs and Hg all have detrimental effects on avian species (Wiemeyer and Porter 1970, Postupalsky 1971, Heath et al. 1972, McClain and Hall

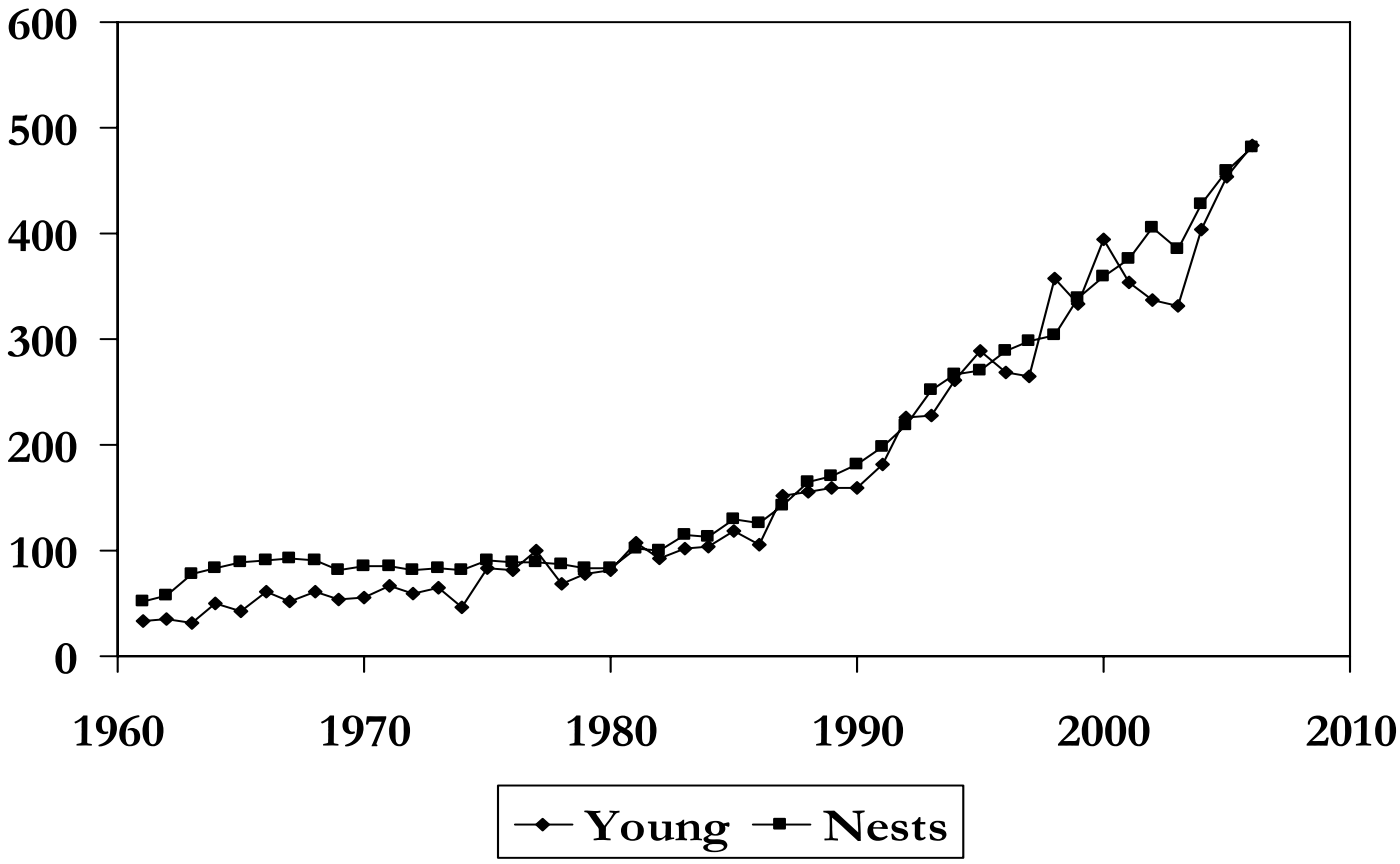

Figure 1. Number of active nests and number of young produced from 1961 to 2006.

1972, Kozie and Anderson 1991, Wiemeyer et al. 1993, Giesy et al. 1994, Bowerman et al. 1998). In Michigan concentrations of Hg are low enough that it is likely that Hg was not a causative effect of population declines (Bowerman et al. 1995). Concentrations of p,p'-DDE and PCBs in Michigan bald eagles have been great enough to have had detrimental effects on production (Postupalsky 1971, Bowerman et al. 1995, Bowerman et al. 1998). Several other organo-chlorines are also found in Michigan bald eagles at very low concentrations and sporadically, they are not suspected to have caused reproductive failures individually (Bowerman et al. 1995). However, the possibility of synergistic effects from combinations of these persistent chemicals is possible (Newton 1979).

Persistent chemicals concentrations have been reported previously in Michigan and were considered to be sufficiently elevated in bald eagle eggs and plasma, to warrant a number of specific recommendations for assessing the widespread contamination (Bowerman et al. 1994, Bowerman et al. 1998, Bowerman et al. 2003). A monitoring program using nestling eagles to track persistent chemical concentrations was established. Nestling bald eagles receive prey items from within the parents' local breeding territory. Concentrations of persistent chemicals in nestling feathers reflect exposure to MeHg, and in nestling plasma reflect exposure to PCBs and pesticides from the food items those nestlings receive, further substantiating the bald eagle as an appropriate bioindicator of ecosystem quality (Bowerman et al. 2002).

DDE and OC PESTICIDES

Field studies have correlated the effects of p,p'-DDE on bald eagle production (Wiemeyer et al. 1993). In several laboratory studies, DDE has been shown to result in eggshell thinning in numerous species (Wiemeyer and Porter 1970, Heath et al. 1972, McClain and Hall 1972, Peakall et al. 1973, Newton 1979). In the shell gland, DDE inhibits the action of carbonic anhydrase which is necessary to supply the carbonate ions used in shell formation (Newton 1979). However the concentrations of DDE and total PCBs are often significantly positively correlated and separation of the effects of DDE from co-occurring toxicants such as PCBs is problematic (Colborn 1991, Wiemeyer et al. 1993). In Michigan as concentration of DDE in bald eagle eggs decreased below the level thought to be necessary to cause embryo lethality due to eggshell thinning the strength of the negative correlation between productivity and concentrations of DDE also decreased, strengthening the theorized correlation. Simultaneously the negative correlation between concentrations of PCBs in bald eagle eggs and productivity became stronger (Bowerman et al. 1995). There is some evidence that PCBs and DDTs have greater effects on breeding in birds whose parents were also feed PCBs and DDTs (Newton 1979).

PCBs

Concentrations of PCBs in the food and eggs of Great Lakes area birds have been suggested as the causative agent for observed declines in productivity of fish eating birds (Giesy et al. 1994). Concentrations of total PCBs in the eggs of bald eagles have also been inversely correlated with productivity (Postupalsky 1971, Kozie and Anderson

1991, Wiemeyer et al. 1993, Bowerman et al. 1998). Concentrations of a PCB congener found in bald eagles eggs were approximately 20 times higher than the lowest toxic concentration tested in American kestrels (*Falco sparverius*) and may have been a factor in the decline of some eagle populations (Hoffman et al. 1998). In the coastal area of the southwestern Baltic Sea a total lack of reproduction in white-tailed sea eagles (*Haliaeetus albicilla*) in the 1960s and 1970s was associated with high concentrations of PCBs (Falandysz et al. 1994). There is some evidence that PCBs have more effect, regardless of concentration, on breeding output with chronic exposure (Newton 1979). While PCB concentrations have been shown to cause reproductive depression and failures alone, they are often positively correlated with DDE concentrations (Mora et al. 1993, Wiemeyer et al. 1993, Bowerman et al. 1995).

Hg

Hg concentrations in eggs of bald eagles have been suggested to have a causative effect on production (Wiemeyer and Porter 1970). However the eggs used in that study also had concentrations of p,p'-DDE greater than the concentration associated with a greater than 50% declines in productivity. Hg can cause neuropathology resulting in changes in behavior, which may disrupt foraging and nesting behaviors (Jagoe et al. 2002). Hg concentrations in eggs have been associated with impaired hatchability and embryonic mortality in a number of bird species (Wiener et al. 2003, Scheuhammer et al. 2007). Reproductive failure and altered nesting behavior in common loons (*Gavia immer*) have been documented (Evers et al. 2008). Laboratory feeding studies have shown acute lethality, neurotoxicity, and altered nesting behavior in northern goshawks

(*Accipiter gentiles*) and red-tailed hawks (*Buteo jamaicensis*) related to Hg concentrations in food (Borg et al. 1970, Fimreite and Karstad 1971, Barr 1986).

MICHIGAN MONITORING PROGRAM

The Michigan Department of Environmental Quality (MDEQ) implemented a monitoring program using the bald eagle to monitor trends of persistent chemicals under the Clean Michigan Initiative in 1999 (MDEQ 1997). These monitored persistent chemicals included PCBs, organochlorine pesticides (OCs), and Hg.

The MDEQ monitoring program was designed such that the data for watersheds would be available prior to the initiation of the National Pollutant Discharge Elimination System (NPDES) permit development and renewal process for each watershed (MDEQ 1997). Consequently, the Michigan bald eagle biosentinel program was on a five year watershed cycle that allowed watersheds to be monitored two to three years prior to the NPDES permit issuance year.

The state was divided into major "watershed years" with 20% of Michigan's watersheds being sampled each year (Figure 2). This sampling procedure allowed for the entire state to be sampled and analyzed every five years. During annual banding activities, blood and feather samples from nestling bald eagles were collected within these designated watersheds. Monitoring contaminant trends at various spatial scales allows for comprehensive assessment of the Great Lakes Basin ecosystem health.

OBJECTIVES

An evaluation of spatial and temporal trends of Hg, PCBs and pesticides in nestling bald eagles of Michigan was conducted. For Hg analysis spatial and temporal

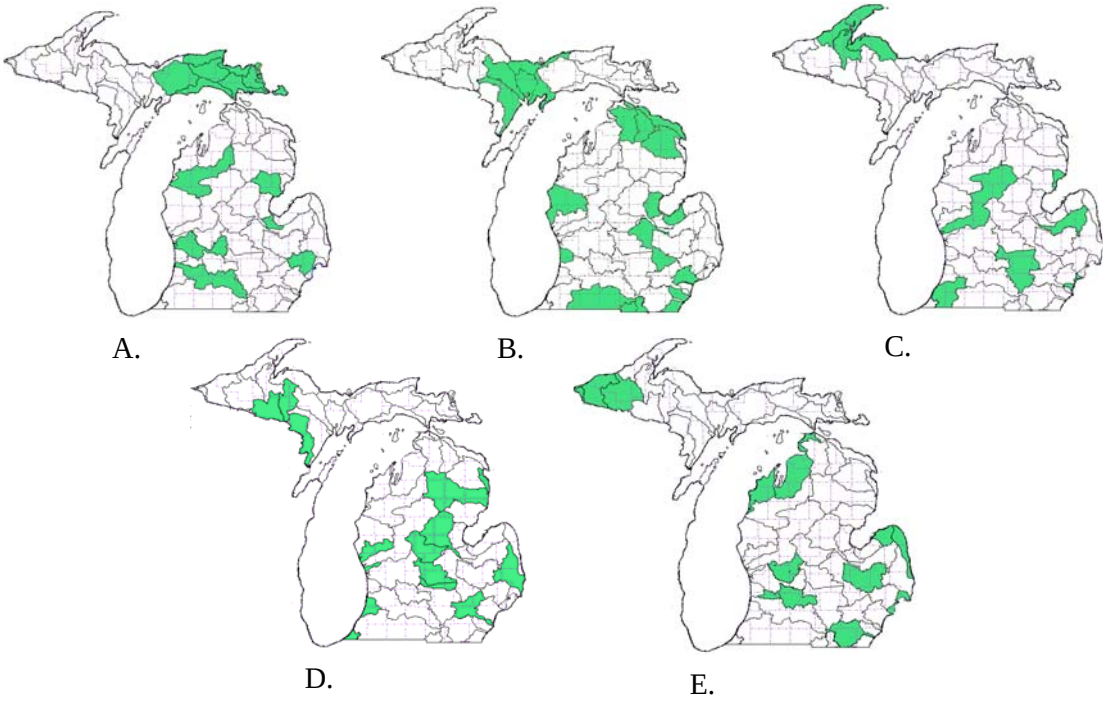

Figure 2. Michigan's watershed delineations and monitoring 'basin years'. A.) 1999, 2004 basin year watersheds (shaded); B.) 2000, 2005 basin year watersheds (shaded); C.) 2001, 2006 basin year watersheds (shaded); D.) 2002, 2007 basin year watersheds (shaded); and E.) 2003, 2008 basin year watersheds (shaded).

trends were determined. For PCBs and pesticides only spatial trends were examined because some data were not available at the time of writing this analysis. As data become available further analysis will be conducted, including temporal trends. Specifically the objectives were:

1. To use concentrations of PCBs in plasma of nestling bald eagles to determine spatial trends of PCBs within the state of Michigan at three spatial scales.
2. To use concentrations of p,p'-DDE in plasma of nestling bald eagles to determine spatial trends of p,p'-DDE within the state of Michigan at three spatial scales.
3. To use concentrations of DDT in plasma of nestling bald eagles to determine spatial trends of DDT within the state of Michigan at three spatial scales.
4. To use concentrations of Hg in feathers of nestling bald eagles to determine spatial trends of Hg within the state of Michigan at four spatial scales.
5. To use concentrations of Hg in feathers of nestling bald eagles to determine temporal trends of Hg within the state of Michigan at five spatial scales.
6. To use concentrations of Hg in feathers of nestling bald eagles to determine statewide temporal trends of Hg among three time periods, 1987-1992, 1999-2003, and 2004-2008

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CHAPTER 1

USING NESTLING BALD EAGLES TO TRACK SPATIAL TRENDS OF PCBS AND PESTICIDES IN AQUATIC ECOSYSTEMS OF MICHIGAN

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is one of the most studied birds of North America. Hundreds of scientific studies have delineated information about its life history, including the influence of various stressors on reproduction (Bowerman et al. 2002). The bald eagle is a large bird of prey and an opportunistic forager, generally preferring fish over a variety of avian, mammalian, and reptilian prey (Buehler 2000). Bald eagles are associated with aquatic habitats (inland watersheds, connecting channels, rivers, and forested shorelines) of North America. Estimates of territory size vary widely based on nesting density, food supply, and method of measurement (Buehler 2000). Bald eagles typically lay one to three eggs per clutch with a mean clutch size of 1.87 (Stalmaster 1987) and both sexes assist in incubation and rearing of young.

The bald eagle population in Michigan has recovered strongly since the population bottle-neck of the 1960s. In the 1960s when Michigan's eagle population was first being monitored less than 100 nests were occupied (i.e., active breeding pairs existed). Today there are approximately 500 occupied nests each year (Figure 1) and over 700 breeding areas in the state. Productivity within each area was determined by dividing the total number of young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Productivity have increased throughout Michigan. Productivity of the 1960s was 0.59 compared to the recent (2000-2006) productivity of

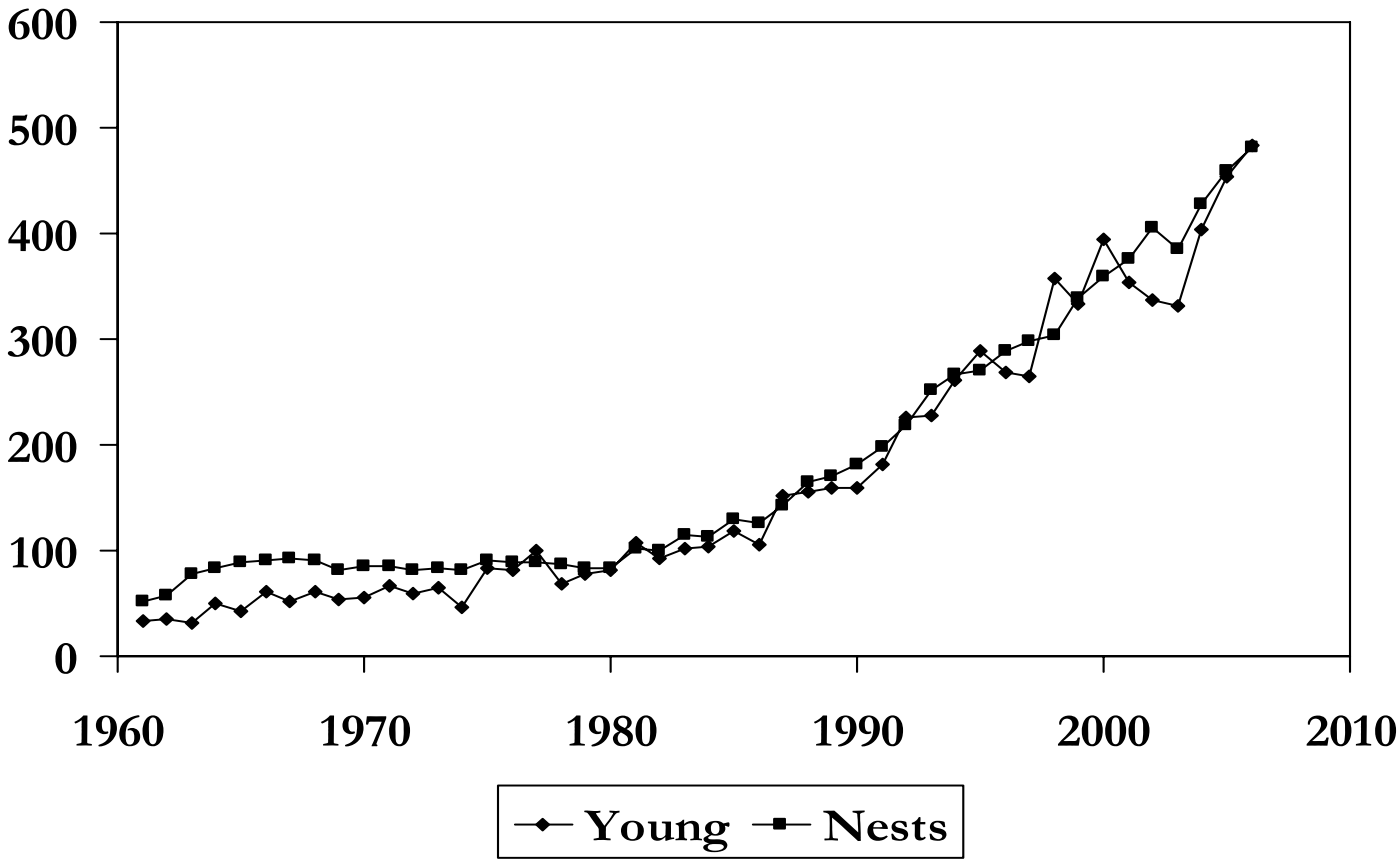

Figure 1. Number of active nests and number of young produced from 1961 to 2006.

0.95. Success was determined by dividing the number of nests producing fledged young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Success have also increased. Success rates of the 1960s were 0.41 compared to recent success rates of 0.62. With increases in population size, Productivity, and Success the number of nestling bald eagle produced each year has also increased. In the 1960s < 50 nestling eagles were produce each year, in recent years (2000-2006) > 400 nestling eagles have been produced each year (Figure 1).

The bald eagle is a tertiary predator of the Great Lakes Basin aquatic food web. Due to it position at the top of the food web, this species is susceptible to biomagnification of a wide array of xenobiotics, including polychlorinated biphenyls (PCBs) and organochlorines (OCs), including dichloro-diphenyl-trichloroethanes (DDT) and its metabolites (DDTs). The bald eagle has been proposed as a biological indicator of exposure to toxic organochlorines and metal compounds for piscivorous wildlife and as a monitor of the effects of contaminant bioaccumulation and biomagnification in the Great Lakes (International Joint Commission 1994, State of the Lake Ecosystem Conference 1998, 2000). Nestling bald eagles receive prey items from within the adult parents' local breeding territory. Concentrations of PCBs and OCs in nestling eagles' blood reflect exposure to these compounds from the food items those nestlings receive, further substantiating the bald eagle as an appropriate bioindicator of ecosystem quality (Bowerman et al. 2002).

The only sources of these organochlorine compounds are anthropogenic. With the exception of PCBs all of these compounds were developed as pesticides. DDT, an

insecticide, was developed by Swiss chemist Paul Hermann Müller who was awarded the Nobel Prize in Physiology or Medicine for its use as a contact poison against several arthropods (Cope et al. 2004). It was used extensively during World War II to combat malaria, lice, and typhus. Post-World War II it was commercialized and used extensively as an agricultural insecticide. DDT was banned in the United States in 1972 and subsequently in many other countries under the Stockholm Convention. Hexachlorocyclohexane (alpha and gamma), heptachlor, heptachlor epoxide, and chlordane (alpha and gamma) were developed as insecticides, while hexachlorobenzene was developed as a fungicide and used in seed treatment. PCBs were commercially produced, to replace flammable mineral oil, as a cooling and insulating fluid for industrial transformers and capacitors. It was also used as a stabilizing additive in flexible polyvinyl chloride (PVC) coatings for electrical wiring and components to enhance the heat and fire resistance of PVC. The majority of these persistent chemicals were synthesized and developed in the 1930s-1940s, increased in usage in the 1950s-1960s, noted as concerns for environmental persistence and biotic accumulation in the 1970s, and banned or controlled in the 1970s throughout North America and Europe (Jones and Voogt 1999).

Persistent chemicals bioaccumulate through aquatic food webs so effectively that the primary exposure pathway for piscivorous wildlife to persistent chemicals is through fish consumption. While persistent toxicant concentrations in many regions of the globe have decreased as a result of bans, several remaining concerns exist. Among these

concerns are the effects on reproduction including egg shell thinning, decreased hatchability, brain deformities, physical deformities, and embryo-lethality.

There have been numerous studies on the detrimental effects of persistent chemicals on different avian species (Cope et al. 2004). Associations between decreased reproductive success and increased concentrations of PCBs and DDTs in eggs and blood of bald eagles have been reported (Wiemeyer et al. 1993, Bowerman et al. 2003, Dykstra et al. 2005, Anthony et al. 2007). Embryos of double-crested cormorant (*Phalacrocorax auritus*) exposed *in ovo* to high concentrations of environmental PCBs were 25 times more likely to have asymmetric brains (Henshel et al. 1997). Ludwig *et al* (1996) documented a high frequency of dead and deformed embryos of double-crested cormorants and Caspian terns (*Sterna caspia*) in the upper Great Lakes in 1986-1991. In general, PCB concentrations are higher in piscivorous avian species than in non-piscivorous birds and higher still in fresh water piscivorous species (Scharenberg 1991b). Concentrations of a PCB congener found in bald eagles eggs were approximately 20 times higher than the lowest toxic concentration tested in American kestrels (*Falco sparverius*) and may be a factor in the decline of some eagle populations (Hoffman et al. 1998). In the coastal area of the southwestern Baltic Sea a total lack of reproduction in white-tailed sea eagles (*Haliaeetus albicilla*) in the 1960s and 1970s was associated with high concentrations of PCBs (Falandysz et al. 1994). While PCB concentrations have been shown to cause reproductive depression and failures alone they are sometimes also correlated with dichlorodiphenyldichloroethylene (DDE) concentrations (Mora et al. 1993).

Of all DDT congeners found in bald eagles p,p'-dichlorodiphenyltrichloroethylene (p,p'-DDE) has received the most attention because of its pervasiveness and demonstrated ecological effects. Bald eagles were shown to have normal young production when egg DDE concentrations were < 3.6 μg/g (wet weight). When egg concentrations were between 3.6 and 6.3 μg/g production was halved and production was halved again when egg concentrations were > 6.3 μg/g (Wiemeyer et al. 1993). In the shell gland, DDE inhibits the action of carbonic anhydrase which is necessary to supply the carbonate ions used in shell formation (Newton 1979). Egg shell thinning due to DDE has had major impacts on populations of bald eagles in the Great Lakes (Wiemeyer et al. 1993, Bowerman et al. 2000). More than 90% of the total DDTs found in plasma of nestling eagles from Michigan is p,p'-DDE (Wierda et al. 2003, Wierda et al. 2005).

Blood is a commonly used to monitor environmental exposure of birds (Olsson et al. 2000, Bowerman et al. 2002, Bowerman et al. 2003, Dykstra et al. 2005). The concentrations of organochlorines in nestling eagles are directly related to the food they receive from the attending adults who hunt within their local breeding territory. Thus, blood from a nestling eagle is an appropriate sample to measure the contamination of the habitat surrounding the nest site. This 'snapshot' of the local contamination allows for easy comparison between different geographic regions and temporal periods. Persistent toxicant concentrations in blood of bald eagles have been previously documented in the Great Lakes region (Bowerman et al. 1998, Bowerman et al. 2000, Dykstra et al. 2001, Bowerman et al. 2002, Bowerman et al. 2003, Dykstra et al. 2005, Parmentier 2006).

The Michigan Department of Environmental Quality (MDEQ) implemented a monitoring program using the bald eagle to monitor trends of a suite of organic pollutants under the Clean Michigan Initiative (MDEQ 1997). These compounds include PCBs, OCs, and mercury. The state has been divided into major "watershed years" with 20% of Michigan's watersheds being sampled each year (Figure 2). During annual banding activities, blood and feather samples from nestling bald eagles were collected within these designated watersheds. This sampling procedure allows for the entire state to be sampled and analyzed every five years. We report here the results of OC and PCB concentrations within plasma of nestling bald eagles from Michigan 2004-2005. The primary objectives of this study were:

1. To use concentrations of PCBs in the plasma of nestling bald eagles to determine spatial trends of PCBs within the state of Michigan at three spatial scales.
2. To use concentrations of p,p'-DDE in the plasma of nestling bald eagles to determine spatial trends of p,p'-DDE within the state of Michigan at three spatial scales.
3. To use concentrations of DDT in the plasma of nestling bald eagles to determine spatial trends of DDT within the state of Michigan at three spatial scales.

METHODS

Study Area

Michigan's geomorphology is classified as Central Lowland plains and is a combination of level to gently rolling lowland and lacustrine plains. Dune fields extend out into the plains along the Great Lakes shorelines. Elevations in the Lower Peninsula

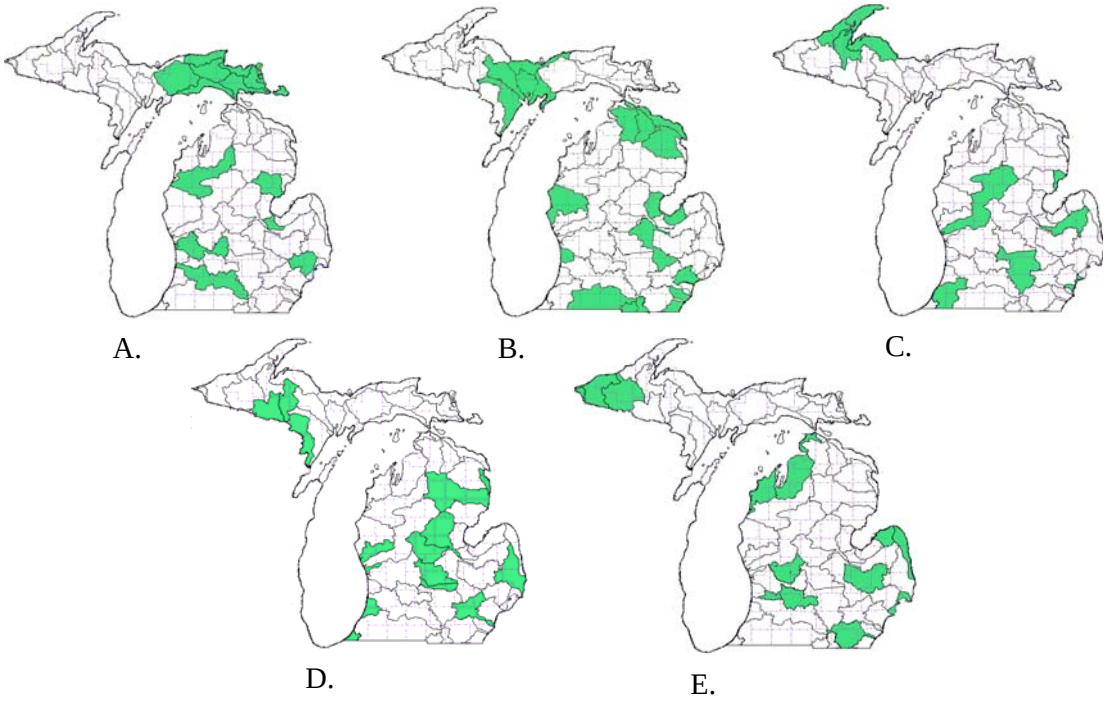

Figure 2. Michigan's watershed delineations and monitoring 'basin years'. A.) 1999, 2004 basin year watersheds (shaded); B.) 2000, 2005 basin year watersheds (shaded); C.) 2001, 2006 basin year watersheds (shaded); D.) 2002, 2007 basin year watersheds (shaded); and E.) 2003, 2008 basin year watersheds (shaded).

of Michigan range from 175-396 m and from 176-256 m in the Upper Peninsula of Michigan. In the Upper Peninsula of Michigan low gradient streams drain into Lakes Superior, Michigan, and Huron. In the Lower Peninsula of Michigan low gradient streams drain into Lakes Michigan, Huron and Erie except in the southern extremity where low gradient streams drain into the Ohio-Mississippi drainages. Small to medium lakes are present but not abundant in the Lower Peninsula of Michigan while numerous lakes and wetlands are found in low lying areas in the Upper Peninsula of Michigan. Wetlands may seasonally flood in low-lying glacial lakebeds (McNab and Avers 1994).

Spatial Analysis

Concentrations of organo chlorine compounds in nestling eagle plasma were compared at three spatial scales: Category; Sub-populations; and Great Lakes Watershed (Bowerman et al. 1994, Roe 2001). Breeding areas, which include all nests used by a territorial pair of eagles, were the sampling unit used for all analyses. The breeding area was assigned to a single grouping at each spatial scale for comparison.

The Category spatial scale compared Inland (IN) and Great Lakes (GL) breeding areas. At all spatial scales which are subdivided into Great Lakes and Inland breeding areas, Great Lakes breeding areas are defined as being within 8.0 km of Great Lakes shorelines and/or along tributaries open to Great Lakes fish runs and inland breeding areas are defined as being greater than 8.0 km from the Great Lakes shorelines and not along tributaries open to Great Lakes fish runs (Bowerman et al. 1994, Roe 2001, Bowerman et al. 2003).

The Subpopulation spatial scale subdivided the Category spatial scale into four GL and two IN groups. The GL subpopulations consisted of Lake Superior (LS), Lake Michigan (LM), Lake Huron (LH), and Lake Erie (LE). The IN subpopulations consisted of Upper Peninsula (UP), and Lower Peninsula (LP).

At the Great Lakes Watershed spatial scale all breeding areas were sorted into eight groupings, based on Great Lakes Basin drainages, four GL and four IN. The GL groups were Lake Superior Great Lakes (LS-GL), Lake Michigan Great Lakes (LM-GL), Lake Huron Great Lakes (LH-GL), and Lake Erie Great Lakes (LE-GL). The IN groups were Lake Huron Inland (LH-IN), Lake Michigan Inland Upper Peninsula (LM-IN-UP), Lake Michigan Inland Lower Peninsula (LM-IN-LP), and Lake Superior Inland (LS-IN).

Aerial Surveys

Aerial surveys were conducted by Michigan Department of Natural Resource (MDNR) pilots and contracted observers to establish which nest within a breeding area was active. An observer on each flight made note of the nest tree species, reproductive status (e.g., eggs, chicks, or adult brooding behavior), and determined location (latitude and longitude) using Global Positioning System units (GPS). The first survey each year was conducted in March or early April to establish nest occupancy. The second aerial survey was conducted in early May to mid June to determine nesting success or failure. If successful, the number of young, stage of development, tree condition, and nest access from the ground were determined. From the observer's notes, field crews were directed to the nests at the appropriate time for sampling. Nestling eagles were sampled at five to

nine-weeks of age, from early May to July each year. Exact nest locations were determined on the ground using GPS.

Field Methods

Eaglet capture

At the nest, a trained crew member climbed the nest tree and secured the nestling eagle(s). Climbers used gaffs, flip ropes, and harnesses to ascend the tree. Once the climber was secure at the nest a nestling eagle was captured, placed in a restraining bag, and lowered to the ground. Nestling eagles were typically captured, restrained, processed and returned to the nest individually. Upon completion of sampling the climber rappelled from the tree.

Sample collection

Processing of nestlings involved collection of blood and morphometric measurements and banding. Nestlings were removed from the restraining bag then placed on their backs with their feet restrained with elastic bandages to avoid injury to the bird or handler. Sterile techniques were used to collect blood from the brachial vein of nestlings. Syringes fitted with 22 gauge x 2.54 cm needles were used for the veinipuncture. Up to 12 ml of blood was drawn from the brachial vein and transferred to heparinized vacuum tubes, and placed on ice in coolers for transfer out of the field. Samples of whole blood were centrifuged within 48 hours of collection and the plasma was decanted and transferred to another vacuum tube and frozen at approximately -20^{o} C for storage. Morphological measurements were collected to determine sex and estimate age of the nestling. Morphological measurements of the culmen, hallux claw, and bill

depth were measured with calipers (Bortolotti 1984a, Bortolotti 1984b, Bortolotti 1984c). The eighth primary feather length and footpad length were measured with a ruler. Procedures developed by Bortolotti (1984b) were used to determine age and sex. After sampling was completed, the nestling eagles were banded with a size 9 U.S. Fish and Wildlife Service (USFWS) rivet band, placed back in the restraining bag, raised, and released to the nest.

From the field, samples were transferred to pre-arranged collection points at various MDNR, U.S. Forest Service, or USFWS field stations. At the end of the sampling effort, all samples were collected and transferred to the USFWS East Lansing Field Office (ELFO), entered into sample storage through a chain-of-custody tracking system, and stored frozen at -20° C. Upon request to the USFWS Chain-of-Custody officer at ELFO, samples were transferred to the Clemson University's Department of Forestry and Natural Resources (CU FNR) for analysis. Capture and sampling methods were conducted according to approved Clemson University Animal Use Protocols (AUP). Handling methods were also approved AUP methods and conducted under USFWS banding permits.

Lab Methods

Organochlorine pesticide extraction

All extractions and analyses were conducted according to procedures detailed in Clemson Institute of Environmental Toxicology (CIET 401-78-01) standard operating procedures. Plasma samples were typically extracted in sets of 19. Chicken plasma was used for laboratory control samples in all analytical batches. In addition to the nestling

eagle plasma samples, each analytical batch contained two chicken plasma matrix spikes, a reagent spike, a reagent blank, and a chicken plasma matrix blank.

Concentrations of organochlorine compounds were quantified by capillary gas chromatography with an electron capture detector using the United States Environmental Protection Agency (EPA) approved methods. All reported results were confirmed by dual column analysis. The quantification limit (QL) for the organic compounds was approximately 2 µg/kg (Table 1). Method validation studies were conducted on chicken plasma as a surrogate matrix to ensure that the data quality objectives of the Quality Assurance Project Plan (CIET 1996, 1999) were met. Average recoveries of 70% -130% for matrix spikes were required under the Quality Assurance Project Plan (CIET 1996, 1999). Correlation coefficients (r^2) for calibration curves consisting of five concentrations of standards were at least > 0.99 for all target analytes in all batches. The average detector response for the instrumental calibration checks was within 20% of the initial calibration for each batch. The average relative percent difference for the spiked analytes in the chicken plasma matrix spike and chicken plasma matrix spike duplicate were less than 30% for all batches.

Statistical Methods

Distributions of contaminant concentrations were tested for normality using the Kolmogorov-Smirnov test and found to be non-normal for both the raw and log-transformed concentrations. Hartley's Fmax test also revealed significantly differing variances between groups. Analyses for differences between multiple groups were therefore conducted using rank converted ANOVAs, a nonparametric test equivalent to

Table 1. Organochlorine contaminant analytes measured in nestling bald eagle blood samples in 2004-2008, with parameter-specific Method Detection Levels (MDLs) and Quantification Levels (QLs).

Organochlorine Contaminant Analyte List	Method Detection Level (MDL)	Quantification Level (QL)
Hexachlorobenzene	0.54	2.01
alpha -Hexachlorocyclohexane	1.94	2.01
gamma -Hexachlorocyclohexane (Lindane)	1.84	2.01
Heptachlor	1.74	2.00
Heptachlor Epoxide	0.77	2.00
alpha -Chlordane	0.75	2.01
gamma -Chlordane	0.55	2.01
Dieldrin	0.97	2.01
2,4'-Dichlorodiphenyldichloroethylene (2,4'-DD	0.86	2.01
4,4'-DDE	0.61	2.01
2,4'-Dichlorodiphenyldichloroethane (2,4'-DDD	1.55	2.01
4,4'-DDD	1.18	2.00
2,4'-Dichlorodiphenyltrichloroethane (2,4'-DDT	1.57	2.01
4,4'-DDT	1.95	2.01
PCB Congener 8	1.94	1.98
PCB Congener 18	1.21	1.98
PCB Congener 28	1.23	1.99
PCB Congener 44	1.52	1.98
PCB Congener 52	0.64	1.98
PCB Congener 66	0.87	2.00
PCB Congener 101	0.38	2.00
PCB Congener 105	1.44	1.98
PCB Congener 110	1.91	2.01
PCB Congener 118	0.58	1.99
PCB Congener 126	0.65	1.99
PCB Congener 128	0.75	1.99
PCB Congener 138	0.65	2.00
PCB Congener 153	0.57	1.99
PCB Congener 156	1.84	2.01
PCB Congener 170	1.28	1.98
PCB Congener 180	1.62	2.00
PCB Congener 187	1.12	1.98
PCB Congener 195	1.03	2.00
PCB Congener 206	1.19	1.98
PCB Congener 209	1.03	1.99

the Kruskal-Wallis test. Because group variances differed significantly for spatial trends post-hoc analyses were conducted using the rank converted Fisher's least significant difference test (LSD). This test is equivalent to the Wilcoxon rank-sum nonparametric analysis. It should be noted that critical values for the Fisher's LSD are set to control only pair-wise error rate and not experiment-wise error rate. This increases the likelihood of detecting a difference at the cost of an increased Type I error-rate. With monitoring as the project's primary function, this was considered to be the preferable compromise between power and Type I error rate because it increases the ability to detect spatial trends of concern as soon as possible.

Though log transformation did not successfully normalize the distribution, concentrations were positively skewed in a manner similar to log-normal distributions commonly seen in other contaminant research. For this reason and in keeping with conventions of environmental toxicology geometric means were included along with medians as indicators of central tendency in the tables provided. Tables also report ranges to facilitate a better understanding of the data presented. All analyses were performed using SAS 9.2 (SAS Institute 2007). An $\alpha = 0.05$ was used to determine statistical significance.

RESULTS

In 2004 and 2005, 159 nestling eagle blood samples were analyzed for PCBs and pesticides. These 159 samples represented 111 breeding areas. Regionally, the analyzed samples were from inland Upper Peninsula (n = 17), inland Lower Peninsula (n = 57),

Lake Superior (n = 14), Lake Michigan (n = 33), Lake Huron (n = 33), and Lake Erie (n = 5) breeding areas. Concentrations that were non-detects are labeled as ND.

Total PCBs

Category

Total PCB concentrations between blood samples from nestling eagles varied significantly at the Category spatial scale ($F = 87.54^{1, 157}$, $p < 0.0001$). Geometric mean Total PCBs concentrations were ranked in the following order from highest to lowest GL (22.34 µg/kg) and IN (ND; Table 2).

Subpopulation

Total PCB concentrations varied significantly among blood samples from nestling eagles at the Subpopulation spatial scale ($F = 19.49^{5, 153}$, $P < 0.0001$). Post-hoc analysis showed that total PCB concentrations in blood samples of nestlings from Lake Erie and Lake Huron were greater than Lake Superior, Inland Lower Peninsula, and Inland Upper Peninsula. Post-hoc analysis also showed that total PCB concentrations in blood samples of nestlings from Lake Michigan and Lake Superior were greater than Inland Upper Peninsula, and Inland Lower Peninsula (LSD = 26.59, d.f. = 153, $p \leq 0.05$). Geometric mean total PCBs concentrations were ranked in the following order from highest to lowest: LE (59.41 µg/kg) , LH (28.85 µg/kg), LM (24.32 µg/kg), LS (7.05 µg/kg), LP (ND), and UP (ND; Table 2).

Great Lakes Watershed

Total PCB concentrations varied significantly among blood samples from nestling eagles at the Great Lakes Watersheds spatial scale ($F = 13.76^{7, 151}$, $P < 0.001$). Post-hoc

analysis showed that total PCB concentrations in blood samples of nestlings from LE-GL were greater than concentrations from LS-GL, LH-IN, LM-IN-UP, LM-IN-LP, and LS-IN. Post-hoc analysis also showed that total PCB concentrations in blood samples of nestlings from LH-GL, LM-GL, and LS-GL were greater than LH-IN, LM-IN-UP, LM-IN-LP, and LS-IN (LSD = 29.96, d.f. = 151, $P \leq 0.05$). Geometric mean total PCBs concentrations for Great Lakes watersheds were ranked in the following order from highest to lowest: LE-GL (59.41 µg/kg), LH-GL (29.56 µg/kg), LM-GL (23.40 µg/kg), LS-GL (7.05 µg/kg), LM-IN-LP (ND), LH-IN (ND), LS-IN (ND), and LM-IN-UP (ND; Table 2).

DDE

Category

DDE concentrations varied significantly between blood samples from nestling eagles at the Category spatial scale ($F = 61.39^{1, 157}$, $P < 0.0001$). Geometric mean DDE concentrations were ranked in the following order from highest to lowest: GL (7.20 µg/kg) and IN (ND; Table 2).

Subpopulation

DDE concentrations varied significantly among blood samples from nestling eagles at the Subpopulation spatial scale ($F = 16.27^{5, 153}$, $P < 0.0001$). Post-hoc analysis showed that DDE concentrations in blood samples of nestlings from LM and LH were greater than concentrations in LE, LS, UP, and LP (LSD = 27.58, d.f. = 153, $P \leq 0.05$). Geometric mean DDE concentrations were ranked in the following order from highest to

lowest: LH (14.14), LE (7.77 µg/kg), LM (6.65 µg/kg), UP (2.03 µg/kg), LS (ND), and LP (ND; Table 2).

Great Lakes Watershed

DDE concentrations varied significantly among blood samples from nestling eagles at the Great Lakes Watersheds spatial scale ($F = 12.28^{7, 151}$, $P < 0.001$). Post-hoc analysis showed DDE concentrations in blood samples of nestlings from LM-GL and LH-GL were greater than concentrations from LE-GL, LS-GL, LM-IN-LP, LM-IN-UP, LH-IN, and LS-IN. Post-hoc analysis also showed that DDE concentrations in blood samples of nestlings from LE-GL and LS-GL were greater than concentrations from LS-IN (LSD = 30.71, d.f. = 151, $P \leq 0.05$). Geometric mean DDE concentrations for Great Lakes watersheds were ranked in the following order from highest to lowest: LH-GL (14.18 µg/kg), LE-GL (7.77 µg/kg), LM-GL (6.31 µg/kg), LM-IN-UP (3.89 µg/kg) , LS-GL (1.00 µg/kg), LM-IN-LP (ND), LS-IN (ND), and LH-IN (ND; Table 2).

Total DDTs

Category

Total DDT concentrations varied significantly between blood samples from nestling eagles at the Category spatial scale ($F = 67.25^{1, 157}$, $P < 0.0001$). Geometric mean total DDT concentrations were ranked in the following order from highest to lowest: GL (12.59 µg/kg) and IN (ND; Table 2).

Subpopulation

Total DDT concentrations varied significantly among blood samples from nestling eagles at the Subpopulation spatial scale ($F = 17.85^{5, 153}$, $P < 0.0001$). Post-hoc

analysis showed that total DDT concentrations in blood samples of nestlings from Lake Michigan were greater than concentrations in Lake Erie, Lake Superior, Inland Upper Peninsula, and Inland Lower Peninsula. Post-hoc analysis also showed that total DDT concentrations in blood samples of nestlings from Lake Huron were greater than concentrations in Lake Superior, Inland Upper Peninsula, and Inland Lower Peninsula. Post-hoc analysis also showed that total DDT concentrations in blood samples of nestlings from Lake Erie were greater than concentrations in Inland Upper Peninsula, and Inland Lower Peninsula. (LSD = 27.09, d.f.= 153, $P \leq 0.05$). Geometric mean total DDT concentrations were ranked in the following order from highest to lowest: LM (20.05 µg/kg), LH (18.13 µg/kg), LE (11.41 µg/kg), UP (4.11 µg/kg), LS (ND), and LP (ND; Table 2).

Great Lakes Watershed

Total DDT concentrations varied significantly among Great Lakes Watersheds ($F = 13.08^{7, 151}$, $p < 0.001$). Post-hoc analysis showed total DDT concentrations in blood samples of nestlings from LM-GL and LH-GL were greater than concentrations from LS-GL, LM-IN-LP, LM-IN-UP, LH-IN, and LS-IN. Post-hoc analysis also showed that concentrations in blood samples of nestlings from LE-GL were greater than concentrations from LM-IN-UP, LH-IN, and, LS-IN (LSD = 30.32, d.f. = 151, $P \leq 0.05$). Geometric mean concentrations of total DDTs were ranked in the following order from highest to lowest: LM-GL (19.94 µg/kg), LH-GL (18.33 µg/kg), LE-GL (11.41 µg/kg), LM-IN-UP (4.02 µg/kg), LS-IN (3.24 µg/kg), LS-GL (ND), LM-IN-LP (ND), and LH-IN (ND; Table 2).

Table 2. Geometric mean (g-mean), median, and range concentration (ppb) of DDT, p,p'-DDE, and PCB in plasma of nestling bald eagles collected within Michigan, 2004-2005. Comparisons were made at 3 geographic scales; Category, Subpopulation, and Great Lakes Watersheds.

Comparison		DDT			p,p'-DDE			PCB		
	N	g-mean	median	range	g-mean	median	range	g-mean	median	range
Category										
Great Lakes	85	12.59	18.28	ND-73.97	7.20	12.71	ND-66.87	22.34	43.88	ND-129.06
Inland	74	ND	4.06	ND-101.63	ND	4.01	ND-67.81	ND	2	ND-544.39
Subpopulation										
Inland Upper Peninsula	16	4.11	3.63	ND-16.77	ND	3.61	ND-16.77	ND	4.22	ND-66.92
Lake Superior	14	ND	7.25	ND-73.97	ND	5.96	ND-66.87	7.05	16.67	ND-258.06
Lake Michigan	33	20.05	20.64	1.00-60.65	6.65	18.78	ND-58.02	24.32	45.77	ND-111.47
Inland Lower Peninsula	57	ND	4.06	ND-101.64	ND	4.02	ND-97.81	ND	2.00	ND-544.39
Lake Huron	33	18.13	20.40	4.19-52.89	14.14	12.71	4.19-41.67	28.85	45.37	ND-163.54
Lake Erie	5	11.41	10.90	4.99-19.39	7.77	8.76	3.99-9.81	59.41	59.72	43.88-95.66
Great Lakes Watershed										
Lake Superior Inland	8	3.24	2.75	ND-15.80	ND	1.00	ND-10.24	ND	1.00	ND-66.91
Lake Michigan Inland Upper Peninsula	6	4.02	5.07	1.00-13.49	3.89	5.07	1.00-13.20	ND	5.00	ND-26.36
Lake Superior Great Lakes	14	ND	7.25	ND-73.97	1.00	5.96	ND-66.87	7.05	16.67	ND-258.06
Lake Michigan Great Lakes	31	19.94	20.64	1.00-60.65	6.31	18.78	ND-58.02	23.40	45.77	ND-111.47
Lake Michigan Inland Lower Peninsula	22	ND	6.00	ND-30.26	ND	6.00	ND-29.37	ND	1.50	ND-55.29
Lake Huron Great Lakes	35	18.33	20.40	4.19-52.89	14.18	12.71	4.19-41.67	29.56	45.36	ND-163.54
Lake Huron Inland	37	ND	4.03	ND-101.63	ND	3.26	ND-97.81	ND	3.1	ND-544.39
Lake Erie Great Lakes.	5	11.41	10.90	4.98-19.39	7.77	8.76	3.98-9.80	59.41	59.72	43.87-95.66

Great Lakes breeding areas are within 8.0km of a Great Lake or along rivers open to Great Lakes fish runs and inland breeding areas are greater than 8.0km from a Great Lake and not along anadromous fish runs.

Other OCs

Neither Hepatachlor nor α-hexachlorocyclohexane were found in nestling plasma samples from 2004 and 2005. Concentrations of Heptachlor-epoxide, hexachlorobenzene, alpha-chlordane, gama-chlrodane, gama-hexachlorocyclohexane, and dieldrin found in nestling plasma but were too low for reliable detection and analysis (Table 2)

DISCUSSION

This study reports the findings of the first two sampling periods of the second 5 year sampling period (2004-2008) of the Michigan Bald Eagle Biosentinel Program (MBEBP). The MBEBP was designed to monitor spatial and temporal trends of persistent chemicals in Michigan's aquatic ecosystem. We do not discuss the temporal trends below because temporal comparisons are made between five year sampling periods.

For all persistent chemicals (i.e., pesticides and PCBs) a general trend was clear, Great Lakes concentrations where higher than inland areas. This is possibly a result of several factors including; location of production of toxicants, patterns of urban, industrial, and agricultural usage, storage practices, and aerial deposition. Most industrial production was located near water sources (e.g., General Electric Hudson Falls plant in Hudson Falls NY). These water sources are often used for cooling of equipment and pre-regulation flushing of equipment. With urban growth there was an increased need for PCB filled industrial transformers and capacitors. Transformers and capacitors can develop leaks through the breakdown of seals and housings, lighting strikes, and fires. If

a transformer/capacitor fire occurred, PCBs could be released into the atmosphere. Pesticides were used extensively in agricultural and urban areas. Michigan's "fruit belt", a highly active agriculture area, is located near Great Lake shorelines, mostly along the Michigan's western shore of Lake Michigan.

The high DDE and total DDT concentrations in the blood of nestling eagles in the western and northern portions of Michigan's Lower Peninsula are likely related to past agricultural and fruit producing industries. At the Subpopulation and Great Lakes Watershed spatial scales Lake Michigan and Lake Michigan Great Lake nestling eagle DDE and total DDTs concentrations were consistently among the highest. Lake Huron and Lake Huron Great Lakes were also high in contamination load concentrations. Michigan's western coast, north eastern portions of the Lower Peninsula, and the "thumb" (i.e., the peninsula east of Saginaw Bay) areas of Michigan have been fruit producers since the decline of the lumber industry. Some of the earliest evidence of the fruit belt in Michigan dates back to 1891 (Garret 2007). Thus with the advance of effective pesticides it is logical to assume they were applied to orchards and farms. Local residents of Michigan have also talked with us in the field, of the days in the 1970s when sprayer trucks would come through neighborhoods spraying DDTs, some people even talk of running in the mist. These practices would likely have occurred in populated urban areas and popular tourist destinations, both of which West Michigan was and is.

In contrast to pesticides, total PCB concentrations were highest in the blood of nestling eagles in Lake Erie and Lake Erie Great Lakes areas. While these results are consistent with previous studies (Bowerman et al. 1998, Bowerman et al. 2003) it is

important to note that the results are based on a small number of nestling blood samples (n = 5) coming from three breeding areas. Because the MBEBP is a Michigan project we have a very small representation of persistent chemicals in Lake Erie.

Spatial trends of total PCBs and DDTs in nestling eagles are similar to trends in whole fish analyzed by the Michigan DEQ Water Quality Bureau (MDEQ-WQB). The MDEQ-WQB monitors temporal trends exclusively. However, a superficial examination of their data comparing total PCB and DDT concentrations of great lakes fish supports the findings of the MBEBP. Average total DDT concentrations in whole fish from MDEQ-WQB monitoring from highest to lowest were: Lake Michigan (0.57 mg/kg), Lake Huron (0.41 mg/kg), Lake Erie (0.23 mg/kg) and Lake Superior (0.13 mg/kg). Average total PCB concentrations in whole fish from MDEQ-WQB monitoring from highest to lowest were: Lake Erie (2.23 mg/kg), Lake Michigan (1.75 mg/kg), Lake Huron (1.59 mg/kg), and Lake Superior (0.026 mg/kg; unpublished data). In nestling eagles Lake Huron is greater than Lake Michigan for total PCBs however the difference in concentrations are small and not statistically significant.

Concentrations of DDE and PCBs have been negatively correlated with bald eagle production (Wiemeyer et al. 1993, Bowerman et al. 1995, Bowerman et al. 1998, Bowerman et al. 2003). DDE has been correlated with egg shell thinning directly through laboratory work and indirectly through biomonitoring work (Wiemeyer and Porter 1970, Heath et al. 1972, McClain and Hall 1972, Bowerman et al. 1995). PCBs have been suggested as the causative agent for observed declines in productivity of fish eating birds (Giesy et al. 1994). Concentrations of total PCBs in the eggs of bald eagles

have also been inversely correlated with productivity (Postupalsky 1971, Kozie and Anderson 1991, Wiemeyer et al. 1993, Bowerman et al. 1998). While p,p'-DDE concentrations have declined they were likely the greatest causative agent of the population declines of the 1960s, however, PCBs are likely the greatest causative agent of reproductive issues in Michigan's eagles today (Bowerman et al. 1995).

The no observable adverse effect limit (NOAEL) for total PCBs in the blood of nestling bald eagles was determined to be 33 μg/kg and 11 μg/kg for DDE (Bowerman et al. 2003). Of the 159 nestling blood samples analyzed for total PCBs 59 (59%) exceeded the NOAEL. Of the 159 nestling blood samples analyzed for DDE 64 (40%) exceeded the NOAEL. It is therefore possible that once these nestlings reach breeding age, they may not be able to reproduce at a level considered to support a healthy population due to elevated DDE or PCB concentrations. The findings that some nestlings have concentrations of 4,4'-DDE and PCBs in their blood above the NOAEL further stresses the importance of the long-term monitoring program to track fluctuations in annual bald eagle productivity with in the state of Michigan.

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CHAPTER 2

USING NESTLING BALD EAGLES TO TRACK SPATIAL AND TEMPORAL TRENDS OF MERCURY IN AQUATIC ECOSYSTEMS OF MICHIGAN

INTRODUCTION

The bald eagle (*Haliaeetus leucocephalus*) is one of the most studied birds of North America. Hundreds of scientific studies have delineated its life history information, including the influence of various stressors on reproduction (Bowerman et al. 2002). The bald eagle is a large bird of prey and an opportunistic forager which generally prefers fish over avian, mammalian, and reptilian prey (Buehler 2000). Bald eagles are associated with aquatic habitats (coastal areas, rivers, lakes, and reservoirs) and forested shorelines of North America. Estimates of territory size vary widely based on nesting density, food supply, and method of measurement (Buehler 2000). Bald eagles lay one to three eggs per clutch with a mean clutch size of 1.87 (Stalmaster 1987) and both sexes assist in incubation and rearing young.

The bald eagle population in Michigan has recovered strongly since the population bottle-neck of the 1960s. In the 1960s when Michigan's eagle population was first being monitored less than 100 nests were occupied (i.e., active breeding pairs existed). Today there are approximately 500 occupied nests each year (Figure 1) and over 700 breeding areas in the state. Productivity within each area was determined by dividing the total number of young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Productivity have increased throughout Michigan.

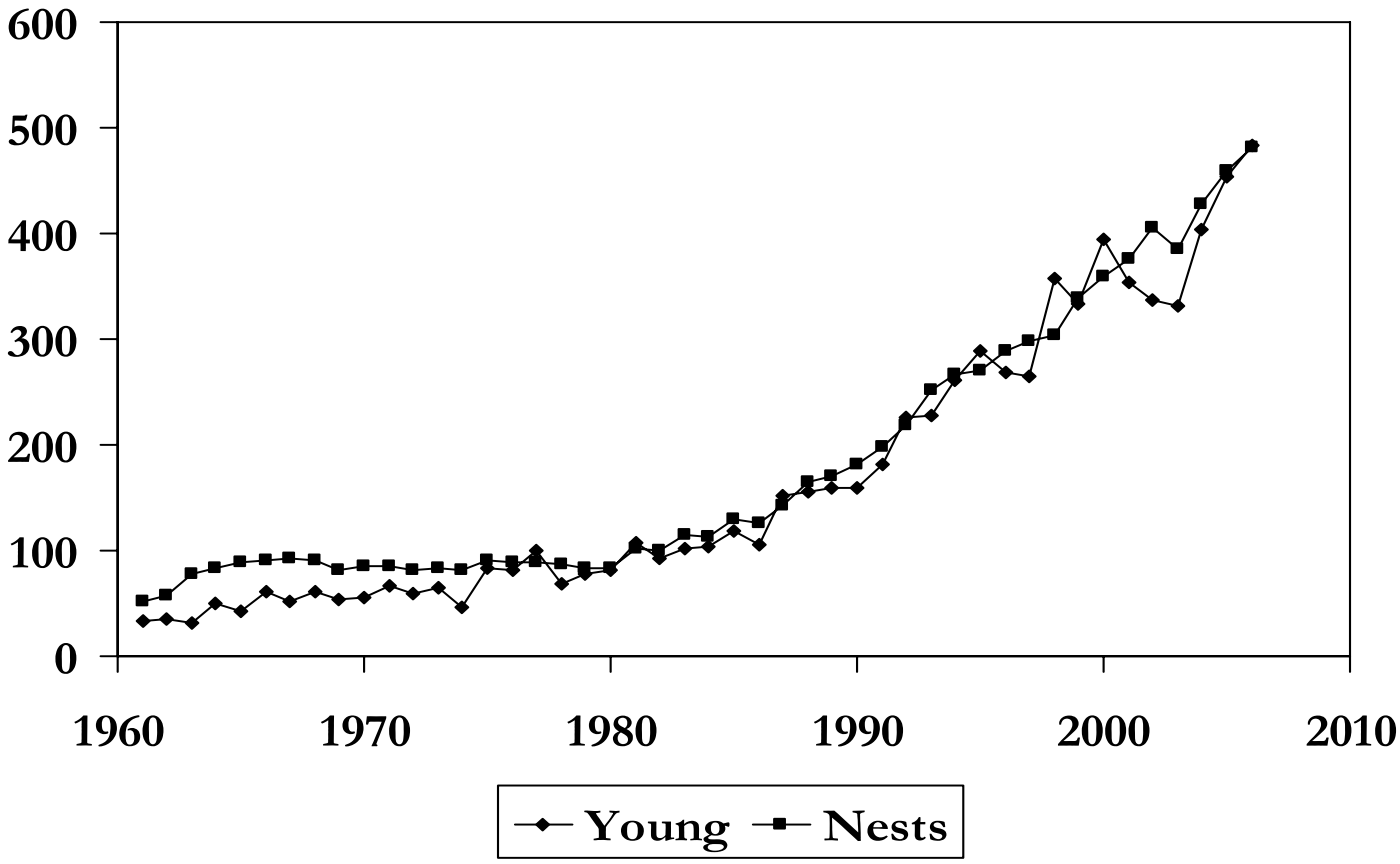

Figure 1. Number of active nests and number of young produced from 1961 to 2006.

Productivity of the 1960s was 0.59 compared to the recent (2000-2006) productivity of 0.95. Success was determined by dividing the number of nests producing fledged young by the number of occupied breeding areas for each year (Postupalsky 1974). Rates of Success have also increased. Success rates of the 1960s were 0.41 compared to recent success rates of 0.62. With increases in population size, Productivity, and Success the number of nestling bald eagle produced each year has also increased. In the 1960s < 50 nestling eagles were produce each year, in recent years (2000-2006) > 400 nestling eagles have been produced each year (Figure 1).

The bald eagle is a tertiary predator of the Great Lakes Basin aquatic food web. Due to its position at the top of the food chain, this species is susceptible to biomagnification of a wide array of xenobiotics, including methylmercury (MeHg). The bald eagle has been proposed as a biological indicator of exposure and effect of aquatic pollutants and is used to monitor the effects of bioaccumulation and biomagnification in the Great Lakes regions (International Joint Commission 1994, State of the Lake Ecosystem Conference 1998, 2000). Nestling bald eagles receive prey items from within the adults local breeding area. Concentrations of MeHg in nestling eagle feathers reflect exposure to MeHg from food items they receive, further substantiating the bald eagle as an appropriate bioindicator of ecosystem quality (Bowerman et al. 2002).

There are many sources of mercury (Hg), both natural and anthropogenic. Natural sources include volcanoes, and mercury deposits. Anthropogenic sources include Hg emissions to the atmosphere which originate from a variety of sources (Harris et al. 2007, SETAC 2007). Hg concentrations in many regions of the globe have increased as a

result of anthropogenic activities. Most of the Hg released into the environment is inorganic, but a small fraction is converted by bacteria to MeHg, a toxic organic compound. Hg is transformed into MeHg when the oxidized or mercuric species (Hg^{2+}), gains a methyl group (CH_3). A variety of microorganisms, particularly methane-producing and sulfate-dependant bacteria are thought to be involved in the conversion of Hg^{2+} to MeHg under anaerobic conditions. Methylation occurs primarily in aquatic, acidic environments with high concentrations of organic matter (Environment Canada 2004). The methylation of Hg^{2+} is primarily a natural, biological process resulting in the production of highly toxic MeHg which bioaccumulates and biomagnifies (Environment Canada 2004). MeHg bioaccumulates through aquatic food webs so effectively that the primary exposure pathway for MeHg in humans and wildlife species is through fish consumption (Harris et al. 2007).

There have been numerous studies on the detrimental effects of Hg on different avian species. Hg can cause neuropathology resulting in changes in behavior, which may disrupt foraging and nesting behaviors (Jagoe et al. 2002). Hg concentrations in eggs have been associated with impaired hatchability and embryonic mortality in a number of bird species (Wiener et al. 2003, Scheuhammer et al. 2007). Reproductive failure and altered nesting behavior have been documented in common loons (*Gavia immer*; Evers et al. 2008). Laboratory feeding studies have shown acute lethality, neurotoxicity, and altered nesting behavior in northern goshawks (*Accipiter gentiles*) and red-tailed hawks (*Buteo jamaicensis*) related to Hg concentrations in food (Borg et al. 1970, Fimreite and Karstad 1971, Barr 1986). In a field study with common loons, adult loons in territories

with greater Hg concentrations left eggs unattended 14% of the time, compared with 1% in territories with lower Hg concentrations (Thompson 1996). In wild birds, environmental MeHg exposure may be associated with a higher potential for infection by disease organisms and decreased growth (Scheuhammer et al. 2007, SETAC 2007).

Feathers are commonly used to monitor environmental exposure of birds to heavy metals (Westermark et al. 1975, Buhler and Norheim 1982, Bruane and Gaskin 1987, Bowerman et al. 1994). Hg is excreted into growing feathers, bound to the feather keratin molecule, and is then relatively stable both physically and chemically (Applequist et al. 1984, Thompson et al. 1998). In birds, about 70% (Honda et al. 1986, Harris et al. 2007) to 93% (Bruane and Gaskin 1987, Harris et al. 2007) of the body burden of Hg is in feathers, and greater than 95% of the Hg in feathers is MeHg (Thompson and Furness 1989, Harris et al. 2007). Hg concentrations in feathers grown after molt are strongly correlated with Hg concentrations in the blood (Evers et al. 2005).

Concentrations of Hg in feathers also reflect concentrations in other tissues. Concentrations of Hg in feathers have been shown to reflect 70-93% of the MeHg concentrations in muscle (SETAC 2007, Burgess and Meyer 2008). Feathers are therefore a relevant tissue for evaluating chronic body burdens (Evers et al. 2005). Hg concentrations in feathers of bald eagles have been previously documented in the Great Lakes region (Bowerman et al. 1994). Atmospheric deposition is considered to be the primary source of Hg accumulating as MeHg in fish inhabiting lakes of the north-central United States (Sorensen et al. 2005). Hg concentrations were considered to be sufficiently elevated in bald eagle feathers from Michigan to warrant a number of specific

recommendations for assessing the widespread Hg contamination problem due to aerially transported Hg loadings (Evans 1993).

The Michigan Department of Environmental Quality (MDEQ) implemented a monitoring program using the bald eagle to monitor trends of a suite of organic pollutants under the Clean Michigan Initiative (MDEQ 1997). These compounds include polychlorinated biphenyls, organochlorine pesticides, and mercury. The state has been divided into major "watershed years" with 20% of Michigan's watersheds being sampled each year (Figure 2). During annual banding activities, blood and feather samples from nestling bald eagles were collected within these designated watersheds. This sampling procedure allows for the entire state to be sampled and analyzed every five years.

The primary objectives of this study were:

1. To use concentrations of Hg in feathers of nestling bald eagles to determine spatial trends of Hg within the state of Michigan at four spatial scales.
2. To use concentrations of Hg in feathers of nestling bald eagles to determine temporal trends of Hg within the state of Michigan at five spatial scales.
3. To use concentrations of Hg in feathers of nestling bald eagles to determine statewide temporal trends of Hg among three time periods, 1987-1992, 1999-2003, and 2004-2008

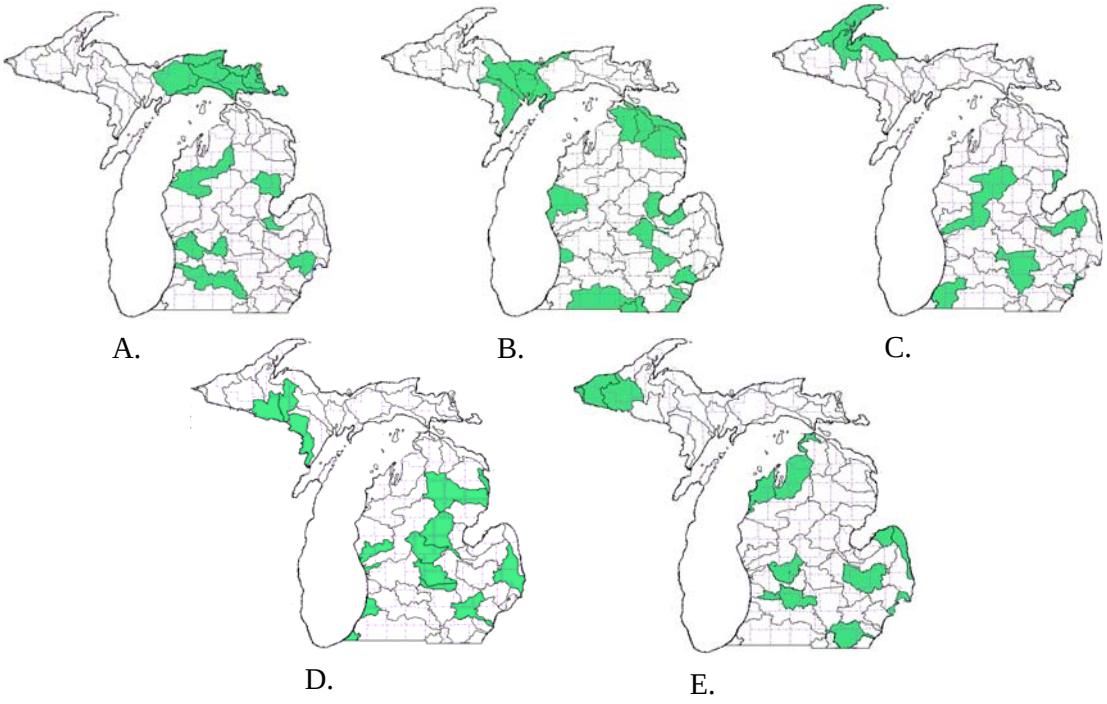

Figure 2. Michigan's watershed delineations and monitoring 'basin years'. A.) 1999, 2004 basin year watersheds (shaded); B.) 2000, 2005 basin year watersheds (shaded); C.) 2001, 2006 basin year watersheds (shaded); D.) 2002, 2007 basin year watersheds (shaded); and E.) 2003, 2008 basin year watersheds (shaded).

METHODS

Study Area

Michigan's geomorphology is classified as Central Lowland plains and is a combination of level to gently rolling lowland and lacustrine plains. Dune fields extend out into the plains along the Great Lakes shorelines. Elevations in the Lower Peninsula of Michigan range from 175-396 m and from 176-256 m in Upper Peninsula of Michigan. In the Upper Peninsula of Michigan low gradient streams drain into Lakes Superior, Michigan, and Huron. In the Lower Peninsula of Michigan low gradient streams drain into Lakes Michigan, Huron and Erie except in the southern extremity where low gradient streams drain into the Ohio-Mississippi drainages. Small to medium lakes are present but not abundant in the Lower Peninsula of Michigan while numerous lakes and wetlands are found in low lying areas in the Upper Peninsula of Michigan. Wetlands may seasonally flood in low-lying glacial lakebeds (McNab and Avers 1994).

Spatial Analysis

Hg concentrations in nestling eagle feathers were compared at four spatial scales: Category; Sub-population; Great Lakes Watershed; and Individual Watershed (Bowerman et al. 1994, Roe 2001). Breeding areas, which include all nests used by a territorial pair of eagles, were the sampling unit used for all analyses. The breeding area was assigned to a single grouping at each spatial scale for comparison.

The Category spatial scale compared Inland (IN) and Great Lakes (GL) breeding areas. At all spatial scales which are subdivided into Great Lakes and Inland breeding areas, Great Lakes breeding areas are defined as being within 8.0 km of Great Lakes

shorelines and/or along tributaries open to Great Lakes fish runs and inland breeding areas are defined as being greater than 8.0 km from the Great Lakes shorelines and not along tributaries open to Great Lakes fish runs (Bowerman et al. 1994, Roe 2001, Bowerman et al. 2003).

The Subpopulation spatial scale subdivided the Category spatial scale into four GL and two IN groups. The GL subpopulations consisted of Lake Superior (LS), Lake Michigan (LM), Lake Huron (LH), and Lake Erie (LE). The IN subpopulations consisted of Upper Peninsula (UP), and Lower Peninsula (LP).

At the Great Lakes Watershed spatial scale all breeding areas were sorted into eight groupings, based on Great Lakes Basin drainages, four GL and four IN. The GL groups were Lake Superior Great Lakes (LS-GL), Lake Michigan Great Lakes (LM-GL), Lake Huron Great Lakes (LH-GL), and Lake Erie Great Lakes (LE-GL). The IN groups were Lake Huron Inland (LH-IN), Lake Michigan Inland Upper Peninsula (LM-IN-UP), Lake Michigan Inland Lower Peninsula (LM-IN-LP), and Lake Superior Inland (LS-IN).

The Individual Watershed spatial scale was defined by Hydrological Unit Codes (HUCs) as defined by the U.S. Geological Survey (USGS). Individual Watersheds were analyzed independently. A second analysis was done by grouping individual watersheds into three types: Great Lakes HUCs (GL-HUCs), Inland HUCs (IN-HUCs), and Mixed HUCs (M-HUCs). These are referred to hereafter as "Grouped HUCs". A GL-HUC was an individual watershed where all breeding areas were previously defined as GL. An IN-HUC was an individual watershed where all breeding areas were previously defined as IN. M-HUCs included both GL and IN breeding areas.

Temporal analyses were conducted to report changes in Hg concentrations over time. Temporal analyses among the three sampling efforts: 1987-1992 (T1), 1999-2003 (T2), and 2004-2008 (T3) were conducted at the state spatial scale. Temporal analyses for Category, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales were conducted between T2 and T3.

Aerial Surveys

Aerial surveys were conducted by Michigan Department of Natural Resource (MDNR) pilots and contracted observers to establish which nest within a breeding area was active. An observer on each flight made note of the nest tree species, reproductive status (e.g., eggs, chicks, or adult brooding behavior), and determined location (latitude and longitude) using Global Positioning System units (GPS). The first survey each year was conducted in March or early April to establish nest occupancy. The second aerial survey was conducted in early May to mid June to determine nesting success or failure. If successful, the number of young, stage of development, tree condition, and nest access from the ground were determined. From the observer's notes, field crews were directed to the nests at the appropriate time for sampling. Nestling eagles were sampled at five to nine-weeks of age, from early May to July each year. Exact nest locations were determined on the ground using GPS.

Field Methods

Nestling eagle capture

At the nest, a trained crew member climbed the nest tree and secured the nestling eagle(s). Climbers used gaffs, flip ropes, and harnesses to ascend the tree. Once the

climber was secure at the nest a nestling eagle was captured, placed in a restraining bag, and lowered to the ground. Nestling eagles were typically captured, restrained, processed and returned to the nest individually. Upon completion of sampling the climber rappelled from the tree.

Sample collection

Processing of nestlings consisted of feather collection and morphometric measurements. Nestlings were removed from the restraining bag then placed on their backs with their feet restrained with elastic bandages to avoid injury to the bird or handler. Three to four feathers were collected from each nestling eagle. Feathers were plucked from the breast area and stored in a small sealed envelope at ambient temperatures. Morphological measurements were collected to determine sex and estimate age of the nestling. Morphological measurements of the culmen, hallux claw, and bill depth were measured with calipers (Bortolotti 1984a, Bortolotti 1984b, Bortolotti 1984c). The eighth primary feather length and footpad length were measured with a ruler. Procedures developed by Bortolotti (1984b) were used to determine age and sex. After sampling was completed, the nestling eagles were banded with a size 9 U.S. Fish and Wildlife Service (USFWS) rivet band, placed back in the restraining bag, raised, and released to the nest. Capture and sampling methods were conducted according to approved Clemson University Animal Use Protocols (AUP). Handling methods were also approved AUP methods and conducted under UWFWS banding permits.

From the field, samples were transferred to pre-arranged collection points at various MDNR, U.S. Forest Service, or USFWS field stations. At the end of the

sampling effort, all samples were collected and transferred to the USFWS East Lansing Field Office (ELFO), entered into sample storage through a chain-of-custody tracking system, and stored at ambient temperature. Upon request to the USFWS Chain-of-Custody officer at ELFO, samples were transferred to Clemson University, Department of Forestry and Natural Resources (CU FNR) for analysis.

Lab Methods

Feather Preparation

Feathers were washed, rinsed, dried, and digested in preparation for Hg analysis. Feathers were placed in a labeled Ziploc® bag containing the detergent Citranox®, agitated, and then rinsed 2 times with nanopure water. Washed feathers were placed in a freezer for 1h and then in a freeze-dryer overnight to remove moisture. The feathers were then weighed and transferred into glass digestion tubes. If the sample was not at least 0.05 g, the sample was not used for Hg analysis. Ten ml of concentrated nitric acid (HNO_3) and sulfuric acid (H_2SO_4; 70:30 v/v) was added to each glass tube which was then covered with a glass marble. Feathers were digested in the tube in a block heater at 80^{o}C for 30 min or until fully digested. The tube was then removed from the block heater to cool for at least 30 minutes; the digestion solution was then transferred to a sealable jar and diluted to 1:20 v/v by adding 190 ml of deionized water. Samples were covered with parafilm, sealed with a cap and stored at room temperature until instrumental analysis.

Mercury Analysis

Mercury analysis followed U.S. EPA Method 245.7 for total Hg by cold vapor Atomic Fluorescence Spectrometer (AFS, Aurora AI 3200). The AFS detector was set at

a wavelength of 237.7 nm and detection limit was reported at less than 1.0 ng/L (Aurora operation manual). The samples were analyzed at the following conditions: gas flow rate = 400ml/min, pump speed = 60 rpm, atomized temperature = 200°C, rinse time = at least 60 sec, uptake time = 60 sec, integration time = 20 sec, 3 duplicates, and reductant = 10% (w/v $SnCl_2$ in 10% (v/v) HCl.

Hg concentrations were estimated and quality assurance and quality control (QA/QC) were maintained with standards and regular equipment detection checks. Hg standards were made using a 1,000 parts per million (mg/kg) +/- 1% Hg standard. Five standards (1, 2, 5, 10, and 20 mg/kg) were made from appropriate ratios of a 100 mg/kg Hg solution and a 10% HCl solution. A standard curve was established from the above standards and after every 5 samples a detection check was performed with either the 5 mg/kg or 10 mg/kg standard. If the detection check was not within 85–115% of the original Hg standard curve, a new standard curve was made and the samples were rerun.

Statistical Methods

Distributions of contaminant concentrations were tested for normality using the Kolmogorov-Smirnov test and found to be non-normal for both the raw and log-transformed concentrations. Hartley's Fmax test also revealed significantly differing variances between groups. Analyses for differences between multiple groups were therefore conducted using rank converted ANOVAs, a nonparametric test equivalent to the Kruskal-Wallis test. Because examinations of temporal trends found that simple linear relationships could not satisfactorily describe the changes in contaminant levels through time and because group variances differed significantly for spatial trends, post-hoc

analyses were conducted using the rank converted Fisher's least significant difference test (LSD). This test is equivalent to the Wilcoxon rank-sum nonparametric analysis. It should be noted that critical values for the Fisher's LSD are set to control only pair-wise error rate and not experiment-wise error rate. This increases the likelihood of detecting a difference at the cost of increasing Type I error-rate as the number of post-hoc comparisons increases. With monitoring as the project's primary function, Fisher's LSD was the preferable compromise between power and Type I error rate for all comparisons except the individual watershed analysis because the number of comparisons was relatively small at these spatial scales and LSD increased the ability to detect spatial and temporal trends of concern. Individual Watershed analysis involved comparisons between 42 watersheds, thus, the more conservative Tukey's test (rank-converted) was used because it includes a correction to control for experiment-wise Type I error rate.

Though log transformation did not successfully normalize the distribution, concentrations were positively skewed in a manner similar to log-normal distributions commonly seen in other contaminant research. For this reason and in keeping with conventions of environmental toxicology geometric means were included along with medians as indicators of central tendency in the tables provided. Tables also report ranges to facilitate a better understanding of the data presented. All analyses were performed using SAS 9.2 (SAS Institute 2007). An $a = 0.05$ was used to determine statistical significance.

RESULTS

Spatial Trends

A total of 424 nestling eagle feather samples, collected from individual nestling eagles from 2004-2008, were analyzed for Hg. These 424 samples represented 226 breeding areas. Comparisons in concentrations of Hg in nestling feathers were made at the Category, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales.

Category

Slight differences in Hg concentrations were observed at the Category spatial scale. No significant differences in Hg concentrations were found between Great Lakes and inland breeding areas ($F = 1.71^{1, 422}$, $P > 0.19$). Geometric mean Hg concentrations were ranked in the following order from highest to lowest: GL (4.65 mg/kg) and IN (4.45 mg/kg; Table 1).

Subpopulation

Hg concentrations varied significantly among feathers from nestling eagles at the Subpopulation spatial scale ($F = 2.53^{5, 418}$, $P = 0.04$). However, post-hoc analysis did not show any significant differences. Geometric mean Hg concentrations were ranked in the following order from highest to lowest: UP (5.85 mg/kg), LS (5.60 mg/kg), LM (4.55 mg/kg), LE (4.21 mg/kg), LH (4.09 mg/kg), and LP (3.71 mg/kg; Table 1).

Great Lakes Watershed

Hg concentrations varied significantly among Great Lakes Watersheds ($F = 2.19^{7, 421}$, $p = 0.03$). Post-hoc analysis showed LS-IN breeding areas were greater than LE-GL

Table 1. Geometric mean (g-mean), median, and range concentration (mg/kg) of mercury in feathers of nestling bald eagle feathers collected within Michigan, 2004-2008. Comparisons were made at 3 geographic scales; Category, Subpopulation, and Great Lakes Watersheds.

Comparison	N	g-mean	median	range
Category				
Great Lakes	220	4.65	5.93	ND-11.04
Inland	204	4.45	6.15	ND-12.15
Subpopulation				
Inland Upper Peninsula	81	5.85	6.34	ND-10.48
Lake Superior	63	5.60	6.19	ND-11.04
Lake Michigan	77	4.55	5.88	ND-8.85
Lake Erie	6	4.21	5.23	ND-6.88
Lake Huron	74	4.09	5.91	ND-10.83
Inland Lower Peninsula	123	3.71	6.04	ND-12.16
Great Lakes Watershed				
Lake Superior Inland	29	6.09	6.43	ND-10.48
Lake Michigan Inland Upper	48	5.99	6.14	1.00-10.34
Lake Superior Great Lakes	63	5.60	6.16	ND-11.04
Lake Huron Inland	81	4.68	5.89	ND-12.15
Lake Michigan Great Lakes	75	4.55	5.88	ND-8.85
Lake Erie Great Lakes.	6	4.21	5.23	ND-6.88
Lake Huron Great Lakes	76	4.10	5.91	ND-10.83
Lake Michigan Inland Lower	44	2.34	6.17	ND-8.80

breeding areas (LSD = 65.38, d.f.=414, $p \leq 0.05$). Geometric mean concentrations of Hg were ranked in the following order from highest to lowest: LS-IN (6.09 mg/kg), LM-IN-UP (5.99 mg/kg), LS-GL (5.60 mg/kg), LH-IN (4.68 mg/kg), LM-GL (4.55 mg/kg), LE-GL (4.21 mg/kg), LH-GL (4.10 mg/kg), and LM-IN-LP (2.34 mg/kg; Table 1).

Individual Watersheds

Hg concentrations varied significantly among Individual Watersheds ($F = 1.43^{42, 381}$, $P < 0.05$). However, post-hoc analysis (Tukey's) did show any significant differences. Hg concentrations for Individual Watersheds ranged from 7.16 mg/kg to 2.25 μg/kg.

Hg concentrations did not vary among Grouped HUCs ($F = 2.30^{2, 822}$, $P > 0.10$). Geometric mean concentrations of Hg for Grouped HUCs were ranked in the following order from highest to lowest: I-HUC (6.11 mg/kg), M-HUC (4.23 mg/kg), and G-HUC (3.95 mg/kg; Table 2).

Temporal Trends

State wide 1987-1992 (T1) vs. 1999-2003 (T2) vs. 2004-2008 (T3)

Hg concentrations varied among T1, T2, and T3 ($F = 28.78^{2, 957}$, $P < 0.0001$). Post-hoc analysis found there were significant differences between all time periods. T1 was significantly greater than T2 and T3. T3 was significantly greater than T2 ($t \geq 1.96$, d.f. = 955, $P \leq 0.05$). Geometric mean Hg concentrations from highest to lowest were T1 (7.44 mg/kg), T3 (4.81 mg/kg), and T2 (3.46 mg/kg; Table 3).

Table 2. Geometric mean (g-mean), median, and range concentration (mg/kg) of mercury in feathers of nestling bald eagles in Michigan 2004-2008 among the Grouped HUCs spatial scale.

Comparison	N	g-mean	median	range
I-HUCs	94	6.11	6.26	1.00-10.48
M-HUCs	257	4.23	5.96	1.00-12.16
G-HUCs	65	3.95	5.90	ND-8.93

Table 3. Geometric mean (g-mean), median, and range concentration (mg/kg) of Hg in feathers of bald eagle nestlings in Michigan, 1987-1992, 1999-2003, and 2004-2008.

Comparison	N	g-mean	median	range
1987-1992	112	7.44	7.90	1.5-18.00
1998-2003	422	3.46	5.05	ND-41.86
2004-2008	424	4.81	6.04	ND-12.16

Analysis of temporal changes T2 vs. T3

The Michigan Bald Eagle Biosentinel Program has now completed two five year cycles (T2 and T3), so comparison of Hg concentrations between these two time periods is important for assessing the utility of the program. While most comparisons within defined subunits within each spatial scale were not significantly different, some differences were observed.

Differences were noted at four spatial scales as well as the Grouped HUC analyses. At the Category spatial scale Hg concentrations were significantly different within GL breeding areas between T2 (geometric mean ($g\bar{x}$) = 3.28 mg/kg) and T3 ($g\bar{x}$ = 4.65 mg/kg; t = -2.05, d.f. = 309.58, p = 0.04; Figure 3). At the Subpopulation spatial scale Hg concentrations were significantly different within UP breeding areas between T2 ($g\bar{x}$ = 2.62 mg/kg) and T3 ($g\bar{x}$ = 5.85 mg/kg; t = -3.39, d.f. = 193.17 P = 0.0008; Figure 3). At the Great Lakes Watershed spatial scale Hg concentrations were significantly different within LM-IN-UP ($g\bar{x}$ = 2.32 and 5.99 mg/kg, t = -2.94, d.f. =127.43, P = 0.0039) and LS-IN (3.21 and 6.09 mg/kg, t = -2.23, d.f. 127.43, P = 0.0304) breeding areas between T2 and T3 (Figure 4). At the Individual Watershed spatial scale Hg concentrations significantly increased within the Keweenaw Peninsula ($g\bar{x}$ = 2.71 and 6.18 mg/kg t = -3.36, d.f. 14, P = 0.0047), Brule ($g\bar{x}$ = 1.10 and 6.32 mg/kg, t = -2.86, d.f. = 28.892, P = 0.0072), Menominee ($g\bar{x}$ = 1.40 and 6.66 mg/kg, t = -2.45, d.f. = 33.246, P = 0.0196), and Shiawassee ($g\bar{x}$ = 0.39 and 4.05 mg/kg, t = -3.36, d.f. = 5.0164, P = 0.0146) watershed breeding areas between T2 and T3 (Table 4, Figure 5). Also, at the

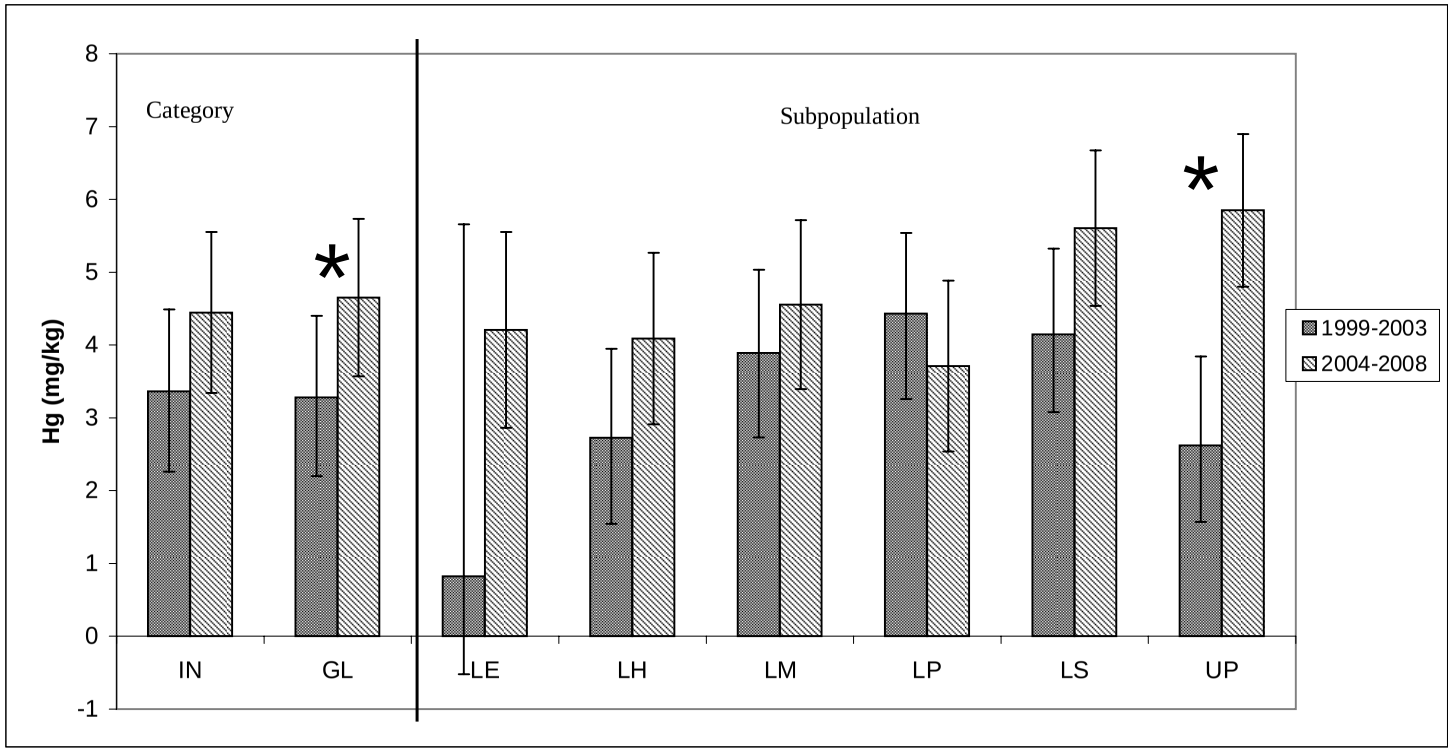

Figure 3. Geometric mean Hg concentrations for feather of nestling bald eagles 1999-2003 and 2004-2008 for Category and Subpopulation spatial scales. Significant differences between time periods are indicated by " * ".

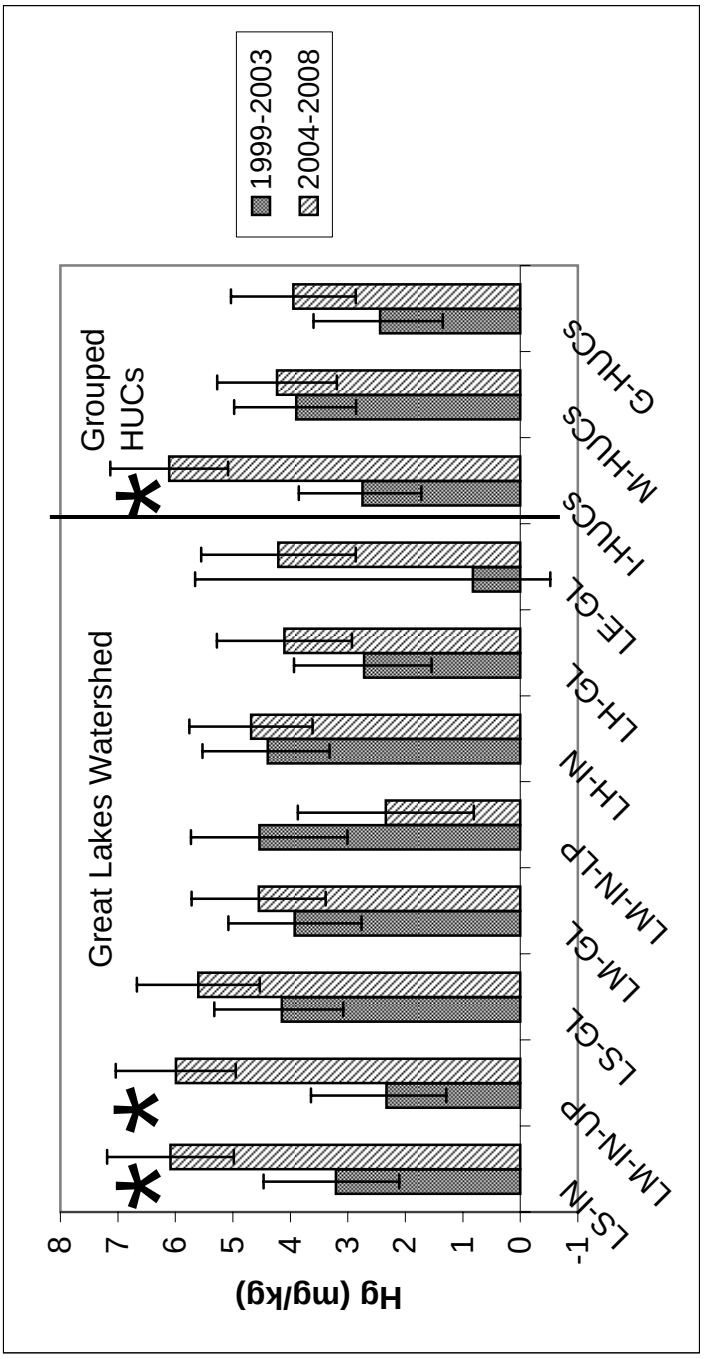

Figure 4. Geometric mean Hg concentrations for feathers of nestling bald eagles 1999-2003 and 2004-2008 for Great Lakes Watersheds and grouped individual watersheds spatial scales. Significant differences between time periods are indicated by " * ".

Table 4. Geometric mean concentrations (mg/kg) of mercury in feathers of nestling eagles and sample size for Individual Watersheds in Michigan, 1999-2003 and 2004-2008. Significant differences between time periods are indicated by " * ".

Watershed	**N**	**1999-2003**	**N**	**2004-2008**
Black-Presque Isle	11	2.17	1	6.38
Ontonagon	20	3.62	6	6.53
Keweenaw Peninsula *	11	2.71	5	6.18
Sturgeon	4	6.86	10	7.16
Dead-Kelsey	20	4.33	24	6.58
Chocolay/Betsy-Two-Hearted	16	5.38	15	5.32
Tahquamenon	2	6.46	8	4.46
Lake Superior Islands	4	2.93	19	5.28
Brule *	21	1.10	12	6.32
Michigamme	12	3.59	3	6.66
Menominee *	24	1.40	19	6.34
Cedar-Ford	10	8.29	5	6.50
Escanaba	8	4.07	1	5.27
Tacoosh-Whitefish	2	11.64	4	3.82
Fishdam-Sturgeon	9	3.87	1	1.29
Kalamazoo	3	5.46	3	0.0022
Lower Grand/Rogue-Flat	1	0.50	5	6.32
Pere Marquette-Pentwater/White	7	2.91	17	6.07
Muskegon	27	3.79	34	2.90
Manistee *	14	8.02	23	3.87
Betsie-Platte	2	4.58	1	6.38
Boardman-Charlevoix	9	2.78	19	5.85
Manistique	17	4.06	11	4.90
Lake Michigan Islands	5	2.35	7	5.97
St. Marys	14	3.30	20	4.76
Carp-Pine	7	4.01	1	1.23
Long Lake-Ocqueoc/Devils Lake-Black	12	3.05	14	6.45
Cheboygan	2	7.70	7	6.29
Black	8	8.71	12	6.16
Thunder Bay	14	5.24	12	6.16
AuSable	36	3.68	30	3.70
AuGres-Rifle/East AuGres	15	3.34	20	4.02
Kawkawlin-Pine	1	4.33	3	5.80
Wiscoggin/Pigeon	7	0.77	3	0.50
Tittabawassee	9	3.03	10	4.68
Shiawassee *	3	0.50	6	4.05

Table 4. cont.

Watershed	Geometric Mean [Hg] ppm (N)			
Cass	6	2.80	6	4.40
Saginaw	1	2.10	3	1.91
Lake Huron Islands	8	5.59	1	5.86
Ottawa-Stony	4	3.15	3	2.89
Upper Wisconsin	5	4.68	2	6.05

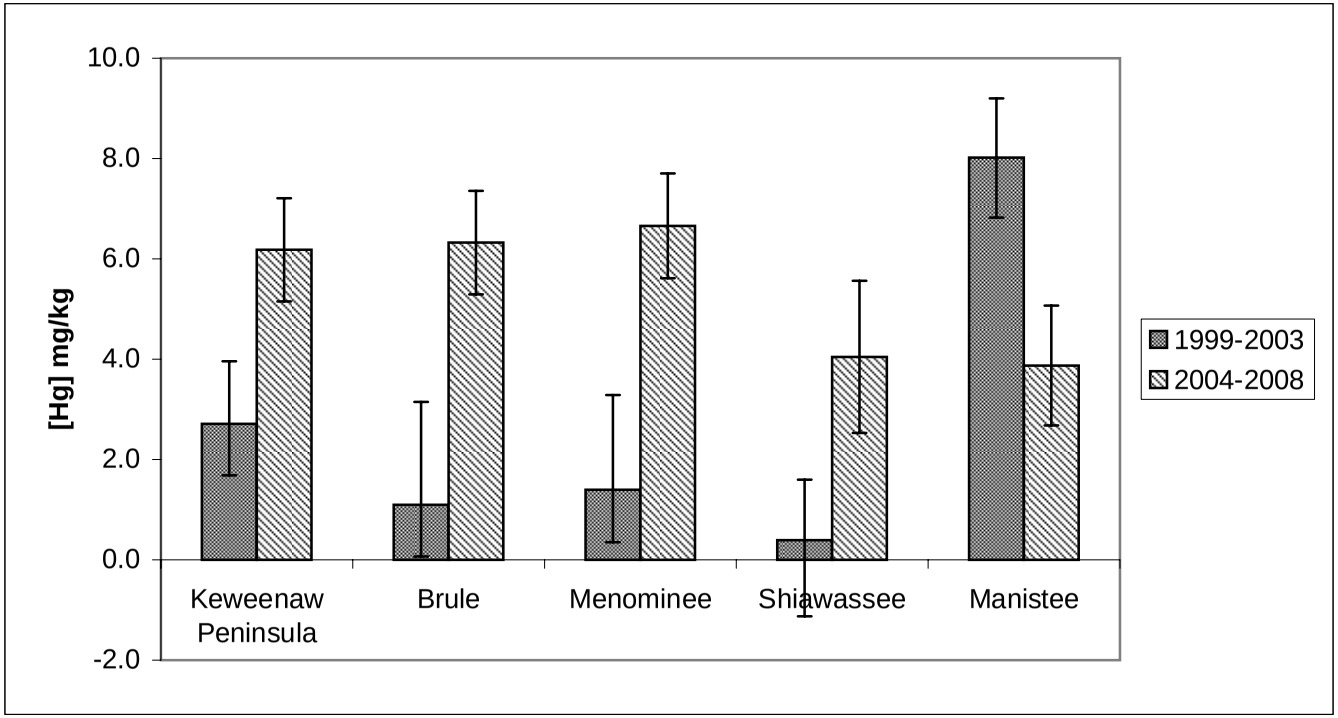

Figure 5. Geometric mean Hg concentrations for feathers of nestling bald eagles 1999-2003 and 2004-2008 for independent individual watersheds spatial scale. Only comparisons resulting in significant differences between time periods are shown.

individual watershed spatial scale Hg concentrations significantly decreased within the Manistee watershed breeding areas between T2 ($g\bar{x}$ = 8.02 mg/kg) and T3 ($g\bar{x}$ = 3.87mg/kg; t = 3.48, d.f. = 35, P = 0.00, Table 4, Figure 5). Grouped HUCs Hg concentrations were significantly different within IN-HUC breeding areas between T2 ($g\bar{x}$ = 2.75 mg/kg) and T3 ($g\bar{x}$ = 6.11 mg/kg; t = -2.87, d.f. = 155.96, P < 0.0046; Figure 4).

DISCUSSION

This study reports the finding of the first two sampling periods of the Michigan Bald Eagle Biosentinel Program (MBEBP). The MBEBP was designed to monitor spatial and temporal trends of Hg in Michigan's aquatic ecosystem. While not part of the MBEBP, an affiliated study using nestling eagles to monitor these trends in the lakes of Voyagers National Park (VNP) in Minnesota is discussed. In addition to trends analysis, the sensitivity of eagles to Hg and their utility as a biosentinel species are discussed.

Spatial Trends

Hg concentrations were highest in the Upper Peninsula of Michigan. At both Subpopulation and Great Lakes Watershed spatial scales the highest concentrations were from the Upper Peninsula. Also, at the grouped individual watersheds spatial scale IN-HUCs (which included UP nests) showed significantly greater Hg concentrations. Elevated Hg concentrations in the feathers of nestling bald eagles from Upper Peninsul of Michigan could be the result of many factors.

Atmospheric deposition of Hg in the Upper Peninsula of Michigan could be heightened as a result of numerous factors. Possible factors include upwind coal consumption in Canada and the north-western United States, and increased consumption in developing countries (e.g., Asia). The open topography of Lake Superior, prevailing winds, and the relief of the western Upper Peninsula may also facilitate the transportations and release of atmospheric Hg.

Locally, large scale environmental changes or environmental characteristics like acid deposition, land use, or climate changes can lead to increased Hg concentrations also local watershed and site conditions can cause large changes in Hg concentration and the ratios of total Hg (tHg) to MeHg. Freshwater aquatic systems associated with wetlands, periodic dry down, and acidic environments are also at greater risk of methylation of mercury (Harris et al. 2007). Blood Hg concentrations in common loons in northern Wisconsin, USA decreased with lake pH (Burgess and Meyer 2008).

At VNP, lakes with dams (Rainy Lake and Crane Lake/Sandpoint) had higher concentration of Hg in fish than lakes without a dam (Kabetogama Lake) (Sorensen et al. 1990). Our results support this where nestling bald eagles had Hg concentrations of 15.1 mg/kg, 13.3 mg/kg and 5.10 mg/kg on Rainy Lake, Crane Lake/Sandpoint, and Kabetogama Lake, respectively. Watershed drainage and flow rates, and water level fluctuations affect Hg transport and residence times, and nutrient and sulfate loading which, in turn, influences Hg methylation and biomagnification potential (Thomsen 2007). The stabilization of water levels by the International Joint Commission resulted in similar decreases in mercury in fish and nestling eagles at VNP (Thomsen 2007).

Temporal Trends

While Hg concentrations are below historic levels they are currently increasing. Slemr et al. (2003) attempted to reconstruct global trends of atmospheric Hg, they reported that Hg concentrations increased in the late 1970s, peaked in the 1980s, and then decreased into the mid 1990s. Mercury concentrations in feathers of nestling eagles in Michigan support this reconstructed trends with a decrease from T1 to T2. This decrease was possibly related to decreased non-point source pollution through the use of cleaner coal and more advanced pollution removal devices (i.e., smoke stack scrubbers). Mercury emissions were also reduced in North America and the European Union between 1990 and 1995.

The current trend of increasing Hg concentrations throughout the state may be a result of increased global consumption of coal, specifically, conspicuous consumption in industrially developing countries (e.g., Asia). Increases in Hg concentrations were seen at several spatial scales when T2 and T3 were compared. The greatest increases were concentrated around the Upper Peninsula and inland breeding areas. As of 1995 the USA produced only 10% of the global mercury emissions and Asia produced greater than 50% (SETAC 2007).

Increases of Hg in nestling bald eagles state-wide coincide with other vertebrate monitoring programs. Increases in Hg concentrations throughout the state of Michigan in

the same time period were observed in fish sampled from 265 lakes and impoundments by the Michigan DEQ Water Quality Bureau (Bohr and VanDusen 2008).

Climate changes could lead to changes in Hg concentrations in Michigan nestling bald eagles. Climate change has been shown to be affecting nesting chronology of bald eagles in Michigan (Bowerman, unpublished data). These changes include earlier laying dates and potential prey base changes. The effects of climate change could alter the bioavailability of Hg to bald eagles and other top-predators due to trophic level changes. These changes could come from shifts in available prey base or environmental changes such as increased frequency and intensity of periodic droughts.

Sensitivity to Hg

No threshold for adverse effects of Hg has been established for bald eagles. Laboratory studies indicated adverse effects including decreased reproduction with Hg levels of 1.5 mg/kg in eggs and 5-40 mg/kg in feathers of multiple species including game birds, waterfowl, and a raptor (Burger and Gochfeld 1997). Burger and Gochfeld (1997) showed that in sparrow hawks (*Accipiter nisus*) feather concentrations of 40 mg/kg resulted in sterility. In common loons, adverse effects levels of 3.0 mg/kg in blood and 40.0 mg/kg in feathers were shown to be correlated with significant decline in reproductive success (Evers et al. 2008).

In our study no breast feathers sampled were greater than 13 mg/kg, much less than the 40.0 mg/kg feather Hg threshold for adverse affects in common loons and sparrow hawks. However, because we were working with nestling eagles who were actively growing feathers our Hg concentrations are more representative of blood levels

(Evers et al. 2005). Thus 88% of the nestling bald eagles sampled would exceed the 3.0 mg/kg blood mercury threshold for common loons (Evers et al. 2005) associated with reproductive impairment or long term effects in loons. Hg concentrations in adult loons can also be up to 10 times greater compared to nestling loons (Evers et al. 2005).

In previous studies which compared adult and nestling feather Hg concentrations from Michigan and Minnesota, adult eagles feathers have been up to 10 times higher than feathers of nestling eagles (Thomsen 2007). If nestling feather concentrations were converted to adult Hg concentrations using a factor of 10 to represent adult exposure, 83% of adults in breeding areas sampled would be above the 40 mg/kg threshold for other avian species. However, no relationships have been observed between Hg concentrations and productivity or success in bald eagles in either study area (Bowerman et al. 1994, Thomsen 2007). It was previously theorized that the life history of adult eagles may have been protective from mercury effects. Since eagles can depurate of up to 90% of their body burden to feathers while they are being replaced, and molting/feather replacement occurs at the same time period as maximum mercury exposure, this may be a protective mechanism for eagles.

Bald eagles may also have a physiological mechanism that allows them to be able to handle a greater insult of Hg by complexing MeHg and Selenium (Se). Bald eagles have been shown to display a greater ability to demethylate MeHg in the brain than common loons (Scheuhammer et al. 2008b). MeHg can be demethylated when complexed with Se. Eagles were shown to have a molar excess of Se while loons had a molar excess of Hg in the brain (Scheuhammer et al. 2008b). This ability to demethylate

MeHg may be why eagles in Michigan can have elevated levels of Hg and have not suffered reproductive declines. Further research may help to understand why eagles appear to not be as sensitive as other avian species.

Utility as a Biosentinel

The MBEBP has now been in effect for two five-year cycles and it is apparent from these results that concentrations of Hg in feathers of nestling eagles is an appropriate measure of Hg exposure in aquatic ecosystems. The Michigan Department of Environmental Quality and Mercury Strategy Staff Report listed the western Upper Peninsula of Michigan as a hot spot (i.e., area of high concentrations) and the Lower Peninsula as having low Hg levels (Kohlhepp 2006). These results are also supported by the MBEBP. The fact that the MBEBP has picked up trends similar to trends reported for fish concentrations, atmospheric deposition, and water quality monitoring speaks to the utility of the project. These 10 years of data in combination with previously collected data from 1987-1992 represents 3 sampling periods of the entire state of Michigan. The trends of decreasing then increasing Hg concentrations over time among many different monitoring programs shows the utility of using bald eagles to monitor the environment. These changes have been observed both spatially and temporally, and therefore, show the utility of the program. With our current knowledge and data base we can now start to focus in on hotspots and monitor these areas more intensively for the effects of Hg.

Recommendations

Based on the results of this analysis of temporal and spatial trends of Hg in aquatic ecosystems by measuring concentrations of Hg in nesting bald eagles, we recommend:

- A more intensive monitoring program for inland and Upper Peninsula breeding areas, and in areas shown to have greater bioavailability of Hg be utilized to investigate the long term effects of Hg on Bald eagle reproductive success.
- Continued monitoring of bald eagle productivity and reproductive success is advisable; if Hg concentrations continue to increase, this project may be in a unique position to observe the threshold at which Hg concentrations start to have detrimental effects on bald eagles.
- Climate change may result in shifts in prey and changing environmental factors, both of which could greatly alter aquatic bioavailability of Hg, therefore it is important that we continue to monitor eagles throughout the state of Michigan to document these impacts.

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In the second study "Using nestling bald eagles to track spatial and temporal trends of mercury in aquatic ecosystems of Michigan." we evaluated Hg concentrations at four spatial scales and three temporal periods. In summary, our study found:

1. Concentrations of Hg in the feathers of nestling eagles were significantly higher in Inland areas with the Upper Peninsula having the highest concentrations
2. At all spatial scales (Category, Subpopulation, Great Lakes Watersheds, and Individual Watersheds) Hg concentrations in the feathers of nestling eagles are increasing.
3. Concentrations of Hg in the feathers of nestling eagles decreased from 1987-1992 to 1999-2003 however, concentrations increased significantly from 1999-2003 to 2004-2008.

The bald eagle has been shown to be an appropriate monitor of Great Lakes environmental quality. Because of its position as a tertiary predator in the Great Lakes aquatic food web it is prone to bioaccumulation of organo chlorines and heavy metals. Furthermore, using nestling bald eagles as biosentinels reduces the possibility of contaminated wintering grounds influencing results. Nestling blood and feather contaminate levels have been shown to be an appropriate method to monitor ecosystem contaminant levels. Both blood and feather samples can be collected during routine nestling banding activities.

In conclusion, measuring the concentrations of both Hg in feathers and organochlorine compounds in plasma of nestling eagles can be used to determine the trends and effects of xenobiotics in aquatic systems in Michigan. With Hg concentrations on the rise, adverse effects including decreased reproduction could occur in bald eagles. The Upper Peninsula of Michigan should be concentrated on because of its characteristics which lead to methylation of Hg. Both PCB and pesticide concentrations for 37% and 40% of the nestling eagles sampled were above the no observable adverse effect level for bald eagles. Thus, it is possible that once these nestlings reach breeding age, they may not be able to reproduce at a level considered to support a healthy population due to elevated concentrations of DDE or PCBs.