

ABSTRACT

Title of Dissertation: **BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*) AS INDICATORS OF GREAT LAKES ECOSYSTEM HEALTH**

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Environmental indicators have been proposed as a means to assess ecological integrity, monitoring both chemical and biological stressors. In this study, we used nestling bald eagles as indicators to quantify direct or indirect tertiary-level contaminant exposure. The spatial and temporal trends of polychlorinated biphenyl (PCB) congeners were evaluated in nestling plasma from 1999–2014. Two hexachlorinated congeners, PCB-138 and 153, were detected with the highest frequency and greatest concentrations throughout Michigan. Less-chlorinated congeners such as PCB-52 and 66 however, comprised a greater percentage of total PCB concentrations in nestlings proximate to urbanized areas, such as along the shorelines of Lake Erie. Toxic equivalents were greatest in the samples collected from nestlings located on Lake Erie, followed by the other Great Lakes spatial regions. Nestling plasma samples were also used to measure concentrations of the most heavily-used group of flame retardants, brominated diphenyl ethers (BDEs), and three groups of alternative

flame retardants, non-BDE Brominated Flame Retardants (NBFRs), Dechloranes, and organophosphate esters (OPs). BDE-47, 99 and 100 contributed the greatest to total BDE concentrations. Concentrations of structurally similar NBFRs found in this study and recent atmospheric studies indicate that they are largely used as replacements to previously used BDE mixtures. A variety of Dechloranes, or derivatives of Mirex and Dechlorane Plus, were measured. Although, measured at lesser concentrations, environmental behavior of these compounds may be similar to mirex and warrant future research in aquatic species. Concentrations of OPs in nestling plasma were two to three orders of magnitude greater than all other groups of flame retardants. In addition to chemical indicators, bald eagles have also been proposed as indicators to identify ecological stressors using population measures that are tied to the fitness of individuals and populations. Using mortality as a population vitality rate, vehicle collisions were found to be the main source of mortality with a greater incidence for females during white-tailed deer (*Odocoileus virginianus*) hunting months and spring snow-melt. Lead poisoning was the second greatest source of mortality, with sources likely due to unretrieved hunter-killed, white-tailed deer carcasses, and possibly exacerbated by density-dependent effects due to the growing population in Michigan.

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GREAT LAKES ECOSYSTEM HEALTH

by

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Dedication

To my family and Joe Gering, for nothing in this world means more to me.

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I attribute every accomplishment in my life to my family. I have learned every ounce of passion, grit, and fortitude through them. They are truly my source of strength, and I could not have completed this dissertation without their unfaltering love and support. Joe Gering has been my source of life, laughter, and happiness. His love, patience, and belief in me is unconditional, even on the most difficult of days. For that, I am forever grateful. His family has also been extremely accepting, caring, and supportive throughout these hectic years.

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We face the question whether a still higher "standard of living" is worth its cost in things natural, wild, and free.

-Aldo Leopold

Table of Contents

| | |
|--|-----|
| Dedication | ii |
| Acknowledgments | iii |
| Table of Contents | v |
| Chapter 1: General Introduction | 1 |
| Figures | 12 |
| Chapter 2: Spatial and Temporal Trends of Polychlorinated Biphenyl Congeners in Michigan Bald Eagles (<i>Haliaeetus leucocephalus</i>) | 14 |
| Introduction | 14 |
| Methods | 17 |
| Field Methods | 17 |
| Laboratory Methods | 18 |
| Statistical Methods | 20 |
| Results | 24 |
| Spatial Patterns | 24 |
| Temporal Trends | 25 |
| Toxic Equivalents | 27 |
| Discussion | 28 |
| Tables | 35 |
| Figures | 42 |
| Chapter 3: Historic and Alternative Flame Retardants in Michigan Bald Eagles (<i>Haliaeetus leucocephalus</i>) | 49 |
| Introduction | 49 |
| Methods | 52 |
| Field Methods | 52 |
| Materials | 55 |
| Analytical Procedures | 56 |
| Statistical Methods | 59 |
| Results | 60 |
| BDEs | 60 |
| NBFRs | 62 |
| Dechloranes | 63 |
| OPs | 65 |
| Discussion | 66 |
| BDEs | 66 |
| NBFRs | 69 |
| Dechloranes | 71 |
| OPs | 76 |
| Tables | 81 |
| Figures | 89 |
| Chapter 4: Historic and Emerging Sources of Mortality in Bald Eagles in Michigan, 1987-2011 | 93 |
| Introduction | 93 |

| | |
|------------------------------|-----|
| Methods..... | 95 |
| Statistical Methods..... | 97 |
| Results..... | 99 |
| Discussion..... | 100 |
| Management Implications..... | 105 |
| Tables..... | 106 |
| Figures..... | 110 |
| Chapter 5: Summary | 113 |
| Bibliography | 119 |

Chapter 1: General Introduction

The Laurentian Great Lakes are the largest system of fresh, surface water on earth, containing about 23,000 km³ of water. The Great Lakes Basin provides about 18% of the world's, and 90% of the United States' supply of freshwater. It also spans an area of 520,000 km² of surface area, with 17,000 km of shoreline and has 5,000 tributaries. Because this water system encompasses such a large area (roughly 41°–51° North latitude and 75°–93° West longitude), physical characteristics vary across the basin. The southern area of the basin is warmer, dominated by deciduous forests, agricultural lands, and urban development. Soils contain a mixture of clay, silts, sand, and gravel. In the North, the climate is cold and terrain consists of granite bedrock (called the Laurentian Shield), topped with a thin layer of acidic soils. Conifers become the main forest species and urban populations are sparse (Canada and Agency 1995).

Outflow of the Great Lakes is less than one percent per year. Lake Superior, for example, has a retention time of 191 years. Because of this and their large surface area, the Great Lakes become a reservoir to a host of contaminants from sources such as runoff from agricultural lands, waste from cities, discharges from industrial areas, leachate from disposal sites, and global atmospheric pollutants (Canada and Agency 1995; Route et al. 2014). Deterioration of water quality and the management of water resources led to the creation of the International Joint Commission (IJC) under the Boundary Waters Treaty between the United States and Canada in 1909. The IJC was given the authority to resolve disputes pertaining to the 1600 km of International

border. By 1964, the IJC began a formal reference study to monitor pollution in the lower Great Lakes. This study laid the rationale for the Great Lakes Water Quality Agreement (GLWQA). The overarching binational goals of the 1978 GLWQA were to restore and maintain the chemical, physical, and biological integrity of the Great Lakes Basin (Freedman and Monson 1989). The GLWQA was further amended in 1987 to identify specific Areas of Concern (AOCs). An AOC is a geographic area in which significant impairment of beneficial uses has occurred due to human activities at the local level. Examples of beneficial use impairments (BUIs) are eutrophication or undesirable algae, restrictions on drinking water consumption or taste and odor problems, and degradation of fish and wildlife populations including loss, deformities, or reproduction problems (Botts and Muldoon 2005; Canada and Agency 1995). Remedial Action Plans (RAPs) are then developed and implemented in order to restore beneficial uses and delist AOCs.

Environmental indicators have been proposed as a means to measure the health of the Great Lakes. Indicators are used to guide the listing/delisting of AOCs, as well as the monitoring of BUIs and effectiveness of RAPs. Chemical indicators are used to assess persistent, bioaccumulating, and toxic substances (PBTs) in biota. These indicators are used to measure temporal and spatial trends of legacy and emerging contaminants of concern throughout the Great Lakes. They are also needed to quantify direct or indirect contaminant exposure via the food chain, affecting the health status of humans, wildlife, or aquatic organisms, and public consumption of fish and wildlife (Commission 2014). Several avian species are currently used as indicators in the Great Lakes. Contaminants have been measured in herring gull

(*Larus argentatus*) eggs from up to 15 breeding colonies. Eggs have been collected from colonies annually over the last four decades to measure legacy POPs and mercury as part of the Environment Canada's Laurentian Great Lakes Herring Gull Monitoring Program (GLHGMP)(Gewurtz et al. 2011; Hebert et al. 2000). More recently, egg pools from several GLHGMP colonies have also been analyzed for an increasing number of chemicals of emerging concern, such as flame retardants (Gauthier et al. 2007; Su et al. 2015). In addition to herring gulls, tree swallows (*Tachycineta bicolor*), belted kingfishers (*Megasceryle alcyon*), and great blue herons (*Ardea herodias*) have been used to monitor contaminants in the Great Lakes region (Bishop et al. 1995; Fredricks et al. 2011; Seston et al. 2010; Seston et al. 2012).

Although selection of a species for monitoring is ultimately dependent on the study's desired purpose, bald eagles have been shown to be effective bioindicators largely due to their high exposure potential, enhanced sensitivity, and low population resilience to lead persistent organic pollutants. This is supported by a previous study ranking the utility and vulnerability of 25 terrestrial vertebrate species as biomonitors of environmental contaminants. Golden and Rattner (2003) developed a utility index to rank the suitability of a species as a sentinel of exposure to a contaminant or class of contaminants (i.e. persistent organic pollutants, cholinesterase-inhibiting pesticides, mercury, lead, or petroleum crude oil). They also developed a vulnerability index to rank susceptibility of populations upon exposure to the same contaminant or class of contaminants. The bald eagle ranked first on the vulnerability index for persistent organic pollutants. Other species with top rankings, such as the double-crested cormorant (*Phalacrocorax auritus*), also fed on higher trophic levels

and had experienced population declines due to organochlorine exposure in the Great Lakes. The bald eagle also ranked third on the utility index, and fifth on the vulnerability index, for lead shot (Golden and Rattner 2003).

Bald eagles (*Haliaeetus leucocephalus*) have been used by the IJC as an indicator species to monitor the spatial and temporal trends of bioavailable contaminants in representative biota throughout the Great Lakes (Best et al. 1990; Bowerman et al. 2003; Gilbertson 1992). Bald eagles are tertiary predators, acquiring containment levels that are representative of those found in the local environment. Average core home ranges for adult nesting bald eagles during the breeding period are approximately 4.9 km²; meaning contaminants consumed by nestlings are limited to the prey sources foraged from proximate watersheds (Bowerman et al. 1998; Bowerman et al. 1995; Watson 2002). In addition to chemical indicators, bald eagles have also been proposed as biological indicators of the abundance and distribution of fish-eating and colonial nesting birds. Biological indicators assess ecological integrity using population matrices that are tied to the fitness of individuals, colonies, and populations of fish-eating birds at multiple geographic scales. The abundance, health, and ability of biological indicators to reproduce reflects the effects of chemical, physical, and ecological stressors within the Great Lakes ecosystem (Commission 2014).

The wealth of existing knowledge regarding life history and habitat preferences also distinguishes bald eagles as an ideal indicator species. Spatially, they are distributed across all five Great Lakes, the connecting channels, and the St. Lawrence River. Temporally, bald eagle reproductive output data has been

continuously monitored for 56 breeding seasons in Michigan, from 1961-2016. These data provide insight for any population level effects caused by environmental contaminants (Bowerman et al. 2003). Bald eagles provided a clear model of the deleterious effects persistent contaminants pose to wildlife when eagle populations across North America severely declined due the teratogenic and eggshell thinning effects of polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and its subsequent metabolites, mainly dichlorodiphenyl-dichloroethylene (*p,p'*-DDE) (Bowerman et al. 1998; Grier 1982).

The Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project in 1999 as an effort to monitor long-term persistent environmental contaminants such as PCBs, DDT, and Mercury in addition to population reproductive data (Bowerman et al. 2002). Through this project, blood and feather samples are collected annually from nestling bald eagles throughout Michigan. The use of blood plasma as a sampling medium allows for the nondestructive collection of samples from year-to-year (Venier et al. 2010). This is especially relevant in nest locations within specific areas, such as AOCs. Historically, eagles nesting along the Great Lakes' shoreline and rivers accessible to Great Lakes' anadromous fish runs have greater concentrations of organochlorine pesticides (*p,p'*-DDE) and PCBs, and impaired productivity, in comparison to eagles nesting in interior areas (Bowerman et al. 1998; Bowerman et al. 2002). Geometric mean concentrations of *p,p'*-DDE and total PCBs in nestling plasma have been shown to be inversely correlated to the productivity and success rates of nesting bald eagles within nine subpopulations of the Michigan Great Lakes watersheds. Concentrations of

PCBs greater than 33 µg/kg, and *p,p'*-DDE greater than 11 µg/kg were associated with a decrease below that of a healthy population productivity level of 1.0 (one young per occupied nest) (Bowerman et al. 2003; Postupalsky 1974; Sprunt et al. 1973). The most recent study analyzing data collected through this project reported that of the 840 nestlings analyzed for total PCBs and DDE, 250 (30%) and 336 (40%), respectively, exceeded the concentrations associated with productivity decreases below that of a healthy population (Wierda et al. 2016).

The Michigan bald eagle population began to increase by the late 1980's following the ban of DDT and PCBs by the Environmental Protection Agency in the 1970's (Grier 1982; Postupalsky 1985). Environmental concentrations of DDE, and its subsequent eggshell thinning effects, were decreasing in Michigan bald eagles (Bowerman et al. 1998). However, relatively high burdens of organochlorine pesticides and PCBs concentrations were reported in nests located along the Great Lakes shorelines (Bowerman et al. 1995; Dykstra et al. 2005). Continued poor nesting success, associated with mutagenic and developmental abnormalities, were observed in nestlings of multiple species of Great Lakes fish-eating birds (Bowerman et al. 1994; Ludwig et al. 1996; Tillitt et al. 1992). The observed effects were referred to as the Great Lakes Embryo Mortality Edema and Deformities Syndrome (GLEMEDS). An epidemiological causation approach linked the syndrome to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), and high concentrations of TCDD-like PCB congeners (Gilbertson et al. 1991). TCDD-like congeners are those that are substituted in the non- and mono-*ortho* positions (Safe 1990; Su et al. 2014b). The unique structure of these compounds enables them to bind to the cytosolic aryl

hydrocarbon (*Ah*) receptor, which mediates many of the toxic responses in fish-eating birds breeding in the Great Lakes including reduced organ and body weight, malformations, and decreased hatchability (Elliott et al. 1996b).

Significant declines in bald eagle productivity (with a threshold productivity level for effects of 0.7 young per occupied nest) have been found at total PCB concentrations above 26 µg/g (fresh wet weight) when measured in 197 salvaged bald eagle eggs (159 clutches) that failed to hatch in Michigan and Ohio from 1986–2000. In addition, eight (11%) of the 73 eggs with a visible embryo exhibited abnormalities prior to 1996, including three with skewed bills. These were not associated with ΣPCB concentrations, and there were no further abnormalities in embryos or nestlings following 1996 (Best et al. 2010). One foot and three bill deformities were also found in nestling eagles in Michigan between 1993 and 1995 (Bowerman et al. 1995; Bowerman et al. 1994). American kestrels have also been shown to be susceptible to *in ovo* concentrations of PCBs, as a significant increase in malformed embryos and hatchlings was observed in eggs dosed with 23,000 pg/g PCB-126 or 2300 pg/g toxic equivalents (TEQs)(Fernie et al. 2003).

Toxic equivalency factors (TEFs), which is an estimate of the potency of the compound in order of magnitude relative to TCDD, can be used with contaminant data to calculate toxic equivalent (TEQ) concentrations (van den Berg et al. 1998). Induction of hepatic cytochrome P4501A (CYP1A) catalytic activity has been used as an effective biomarker of exposure to TCDD-like compounds. Based on the results of 25 bald eggs that were collected in British Columbia in 1992 then allowed to hatch and monitored for 24 hours, hepatic CYP1A induction as a biomarker occurred at

whole egg TEQ concentrations of 210 ng/kg ww in bald eagle chicks (Elliott et al. 1996b). While reporting concentrations of Σ PCBs can give an estimate of absolute exposure, it does not allow for the calculation of TEQs with congeners with the greater potential to cause adverse effects, such as those with non- and mono-*ortho* substitutions. In addition, spatial and temporal analyses of individual congeners give insight into the persistence, distribution, and bioavailability of each congener depending on degree of chlorination.

In addition to legacy contaminants of concern, bald eagles can be used as indicators of chemicals of emerging concern. Flame retardants are becoming increasingly ubiquitous in the environment, detected in air, sediment, and biota of the Great Lakes (Su et al. 2015; Venier et al. 2015; Venier et al. 2010; Yang et al. 2012). For several decades, flame retardants have been added to manufactured materials such as plastics, foams, wire coatings, textiles, and furniture to delay ignition of fires and reduce flammability (Covaci et al. 2011; van der Veen and de Boer 2012). Following the overwhelming scientific evidence of their occurrence and concern as environmental pollutants, the flame retardant industry voluntarily halted the production and sale of the most frequently-detected group of flame retardants, brominated diphenyl ethers (BDEs) (Jones and De Voogt 1999; Route et al. 2014). To replace BDEs, flame retardant industries are using unregulated alternative flame retardants. The penta-BDE commercial mixture (consisting of five bromine substitutions) for example, was largely replaced by Firemaster 550, which contains two non-BDE brominated flame retardants (NBFRs), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate

(TBPH)(Ma et al. 2011). In addition, decabromodiphenyl ether (deca-BDE, or BDE-209) was phased out in the United States in 2013 and replaced by decabromodiphenyl ethane (DBDPE) (EPA 2009; Venier et al. 2012).

Other compounds that have been used as minimal components in flame retardant mixtures for several decades may become more widely used as brominated compounds are taken off the market. One group of these compounds are dechloranes, or highly chlorinated norbornene flame retardants, that have been detected in air and sediment of the Great Lakes (Qiu et al. 2007; Venier et al. 2015; Yang et al. 2011). Organophosphate esters (OPs) are another class of non-brominated replacement flame retardants that are likely to increase in usage (Venier et al. 2015). Atmospheric concentrations of total OP flame retardants were about 100, 1,200, and 600 times greater (on average) than total BDE, TBB, and TBPH, concentrations, respectively, at five sites on Lakes Superior, Michigan, and Erie in 2012 (Salamova et al. 2014). Nine OPs were also detected in at least one of 115 herring gulls collected throughout the Great Lakes in 2012 (Su et al. 2015).

A few studies have used bald eagles as indicators to monitor concentrations of BDEs (Dykstra et al. 2005; Route et al. 2014; Venier et al. 2010). Only one study has analyzed alternative flame retardants in Great Lakes' eagles however, reporting detections of only two NBFR compounds and one dechlorane compound (Venier et al. 2010). The long-term collection of samples through the Michigan Bald Eagle Biomonitoring Project allows for the use of archived plasma samples for retrospective analyses of compounds of emerging concern such as flame retardants.

Bald eagles have previously been used as biological indicators of the health of Great Lakes fish-eating birds. As biological indicators, the use of population matrices, abundance estimates, and individual health assessments provide insight into effects of chemical, physical, and ecological stressors within the Great Lakes ecosystem. Michigan bald eagle populations have recovered from a low of 52 breeding pairs in 1961 to 786 in 2015 (Figure 1.1). Productivity is defined as the number of fledged young per occupied nest (Postupalsky 1985). The goal of all Federal Recovery Plans for bald eagles was a productivity of 1.0. Productivity for the eagles nesting in Michigan increased from a low of 0.40 young per occupied nest in 1963, to maintaining levels around 1.0 (Figure 1.2). As the number of breeding eagles continues to grow in Michigan, the ongoing statewide collection of population data contributes to the better understanding of emerging sources of environmental and anthropogenic stressors.

Mortality is a common population vital rate used to assess population turnover, stability, and the vulnerability of different age groups or sexes within subpopulations (Newton 1979). Counts of eagle mortality have been used to identify risks posed to not only fish-eating birds, but a wide range of predatory and scavenging birds as well. A few anthropogenic stressors or risks include anticoagulant rodenticide and barbiturate poisoning, vehicle collision-related trauma, lead intoxication, and electrocution (Harris and Sleeman 2007; Kelly et al. 2014; Russell and Franson 2014). Lead toxicosis, in particular, has been a growing concern due a number of recent studies reporting it as a major source of scavenger mortality (Franson and Russell 2014; Nadjafzadeh et al. 2013; Russell and Franson 2014;

Warner et al. 2014). Historically, lead toxicosis in bald eagles was a direct result of the ingestion of lead shot from dead or wounded waterfowl, and was a major factor leading to the banning of lead shot for waterfowl hunting in 1999 (Friend et al. 2009; Kendall et al. 1996). Current research however, has linked lead toxicosis in bald eagles to ingested lead fragments embedded in tissues or offal of lost or discarded upland and large game animals (Stauber et al. 2010; Warner et al. 2014). As Michigan bald eagle populations increase, their reliance on the terrestrial prey sources, and subsequent mortality events relating to these sources, may also increase. The overarching objective of this study is to utilize bald eagles, using samples collected through the Michigan Bald Eagle Biomonitoring Project, as chemical and biological indicators of the Great Lakes basin. Nestling plasma will be used to determine spatial and temporal patterns of PCB congeners from 1999-2014. This study will also retrospectively analyze nestling plasma samples to determine the presence and concentrations of both well-studied flame retardants, and those of emerging concern from 2000-2012. Lastly, this study will evaluate the major sources of bald eagle mortality or grounding, and subsequent confounding factors because of an increasing population.

Figures

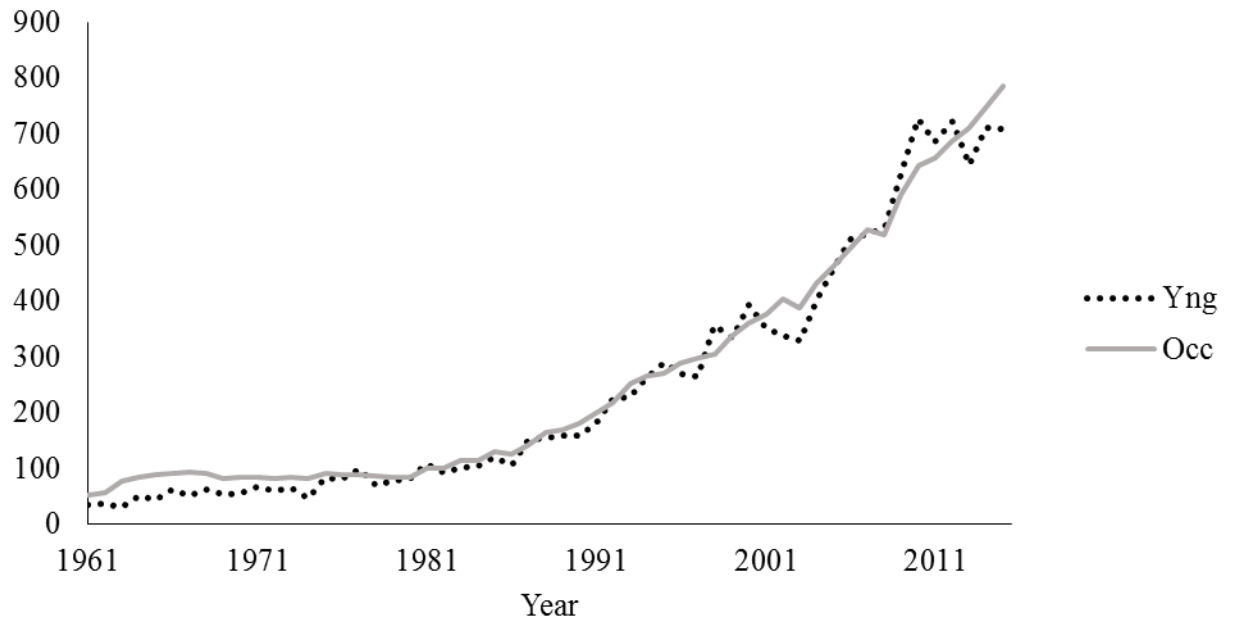


Figure 1.1 Bald eagle (*Haliaeetus leucocephalus*) number of fledged young (Yng) and occupied nests (Occ) in Michigan from 1961 to 2015.

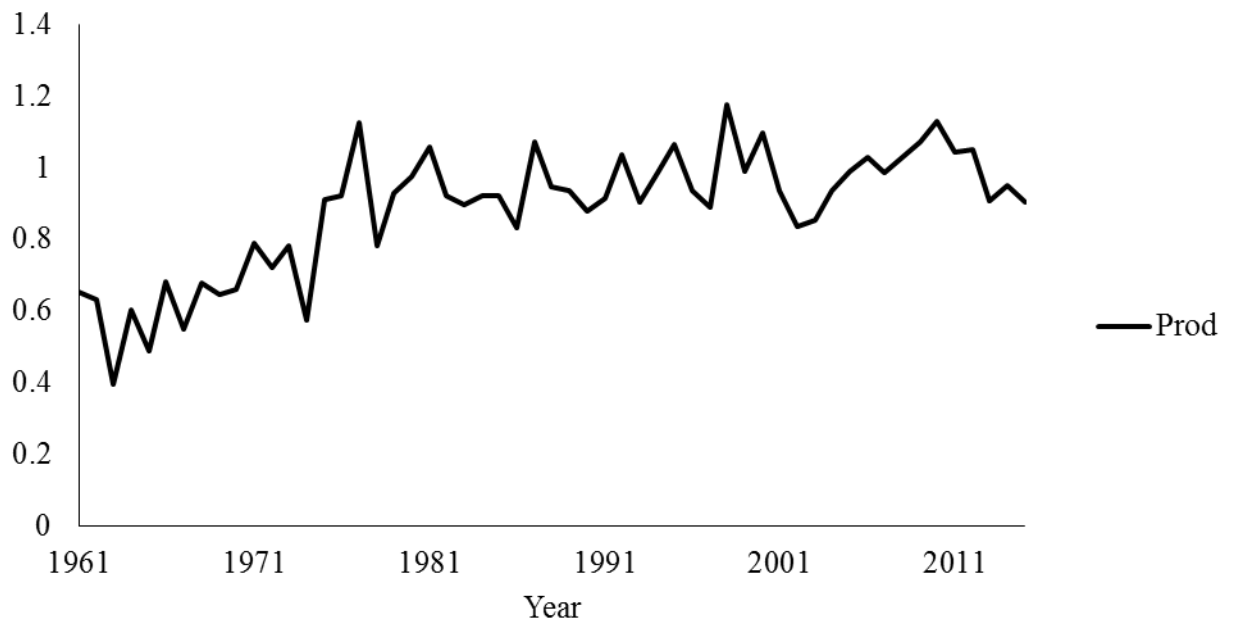


Figure 1.2 Bald eagle (*Haliaeetus leucocephalus*) productivity (young per occupied nest; Prod) in Michigan from 1961 to 2015.

Chapter 2: Spatial and Temporal Trends of Polychlorinated Biphenyl Congeners in Michigan Bald Eagles (*Haliaeetus leucocephalus*)

Introduction

Polychlorinated biphenyls (PCBs) are a class of synthetic chlorinated hydrocarbon chemicals known for low electrical conductivity and high resistance to thermal breakdown. Because of their chemical stability, PCBs have been widely used since the 1930's as dielectric fluids in capacitors and transformers, flame retardants, and plasticizers. PCBs are one of the 12 groups of persistent organic pollutants (POPs) recognized in the Stockholm Convention on POPs (Nyberg et al. 2014), and are found throughout the environment despite being banned in the United States by the Environmental Protection Agency in 1979.

PCB distribution throughout the environment is highly dependent on the degree of chlorine substitution. Volatilization and atmospheric transport is a major pathway for less chlorinated congeners due to their greater solubility and vapor pressure (Health and Services 2000). Higher chlorinated congeners exhibit a greater octanol/ water partition coefficient and therefore, a greater tendency to sorb to sediments, organic matter, and effectively accumulate within biota in aquatic environments. Because of this, aquatic sediments can act as a substantial reservoir, slowly releasing PCBs over time (Hoffman 1995). PCB toxicity and bioaccumulation are structure dependent, and species-specific. Congeners that are substituted in the non- and mono-*ortho* positions are considered to behave similar to 2,3,7,8-

tetrachlorodibenzo-*p*-dioxin (TCDD)(Safe 1990; Su et al. 2014b). The unique structure of these compounds enables them to bind to the cytosolic *Ah*-receptor, which mediates many of the toxic responses in fish-eating birds breeding in the Great Lakes including reduced organ and body weight, malformations, and decreased hatchability (Elliott et al. 1996a). These observed developmental abnormalities were referred to as GLEMEDS (Great Lakes Embryo Mortality Edema and Deformities Syndrome)(Gilbertson et al. 1991). Despite the general improvements in bald eagle productivity throughout North America following the ban of dichlorodiphenyltrichloroethane (DDT) in the 1970's, GLEMEDS contributed to the poor breeding success of bald eagles in certain regions of elevated PCB concentrations in the late 1980's and 1990's (Anthony et al. 1993; Best et al. 2010; Bowerman et al. 1994). Total PCB concentrations of 2.5 µg/g or greater in bald eagle eggs have been positively correlated with decreases in bald eagle productivity below one young per occupied nest, the level needed to maintain a healthy population (Bowerman et al. 2003; Wiemeyer et al. 1993). Risk assessments of PCBs for a species in a given region is difficult however, as congeners occur in mixtures which change over time due to weathering, differential accumulation, and metabolism (Su et al. 2014b). Toxic equivalency factor (TEF), which is an estimate of the potency of the compound in order of magnitude relative to TCDD, can be used with contaminant data to calculate toxic equivalent (TEQ) concentrations (van den Berg et al. 1998). Therefore the TEQ, or summed values of the individual congeners times their TEFs, are a useful predictive tool as they are a response measure for all compounds, and their interactions in a mixture (Koistinen et al. 1997).

PCBs tend to biomagnify to a higher degree in aquatic ecosystems, resulting in greater exposure levels for aquatic species in comparison to terrestrial (Vander Zanden and Rasmussen 1996). In addition, PCB concentrations positively increase with species trophic position due to their highly lipophilic nature (Brázová et al. 2012; Custer et al. 2010; Elliott 2005; Elliott et al. 2009). Sea eagles (Genus *Haliaeetus*) are tertiary predators, feeding opportunistically on aquatic food chains and have been extensively used as indicators of ecosystem health in the Laurentian Great Lakes (Bowerman et al. 1998; Bowerman et al. 2002; Dykstra et al. 1998; Dykstra et al. 2001; Dykstra et al. 2010; Elliott and Norstrom 1998; Route et al. 2014; Venier et al. 2010). A considerable range of contaminant levels may be measured between individual sea eagles within a given region due to spatial variation in environmental contaminant concentration or differences in foraging trophic level (Donaldson et al. 1999; Elliott et al. 2009; Gill and Elliott 2003; Helander et al. 2008). Although PCB concentrations have been decreasing in the Great Lakes since the early-1970s (Hebert et al. 1999; Lamon et al. 2000; Pekarik and Weseloh 1999; Stow et al. 1995), monitoring temporal trends in a given region is important to determine the balance between inputs and loss processes. Time-trends of specific congeners, rather than simply total PCB calculations, can also give greater insight into fate and persistence rates of change (Nyberg et al. 2014).

PCBs have been measured in bald eagles from 1999–2014 through the Wildlife Biosentinel Monitoring Project that was implemented by the Michigan Department of Environmental Quality. Through this project, plasma samples are collected from 6 to 9 week old nestlings throughout Michigan and measured for a

suite of organochlorines and metals. Although concentrations have been decreasing, this project has shown that PCBs contribute to decreased bald eagle productivity, with significant declines observed in eggs above 26 $\mu\text{g/g}$ ΣPCBs (fresh wet wt) (Best et al. 2010). This study aims to use bald eagle plasma samples collected from 1999–2014 to evaluate (1) the spatial differences among PCB congeners in Michigan, (2) the temporal trends of congeners most frequently detected in Michigan, and (3) Differences in TEQ concentrations among spatial regions in Michigan.

Methods

Field Methods

Aerial surveys were flown twice a year to 1) determine occupied nests, defined by the presence of adult birds, and 2) determine productivity, or the number of young produced per occupied nest (Postupalsky 1974). We then visited productive nests and temporarily removed the nestlings to draw up to 12mL of whole blood, collect morphometric measurements for age and sex calculations (Bortolotti 1984a; Bortolotti 1984b; Bowerman et al. 1995). We completed all field procedures in accordance with the Animal Use Protocols of Clemson University (30067 & AUP2009-005) and the University of Maryland (744587-2), as well as the United States Geological Survey Bird Banding permit, and scientific collecting permits of the United States Fish and Wildlife Service and the Michigan Department of Natural Resources. Whole blood was refrigerated for no more than 48hours before it was centrifuged and plasma was pipetted into separate glass tubes for storage at

approximately -20°C. When nests contained multiple nestlings in a single year, we randomly selected one plasma sample for the extraction process.

Laboratory Methods

We extracted PCB compounds from bald eagle nestling plasma using the solid-phase micro extraction procedure described in (Sundberg et al. 2006). Twenty nestling plasma samples (0.1 mL) were run in each set, along with two samples of chicken (*Gallus domesticus*) plasma (0.1 mL) to be used as a control. Three surrogate standards, PCB-103, Tetrachloro-m-xylene (TCMX), and 4,4'-Dibromooctafluorobiphenyl (DBOBF) were added to these samples to assess consistency in the extraction process. Two internal standards, 1-Bromo-2-nitrobenzene (BrNB) and 2,2',4,4',5,5'-Hexabromobiphenyl (HBB), were added to the samples following extraction to assess concentration consistency. All standards were purchased from Ultra Scientific (North Kingstown, RI). Two more sterile chicken plasma samples (0.1 mL) were fortified with surrogate compounds and known amounts of all analytes of interest (spikes) to examine recovery from the extraction and cleanup process.

We added 8M solid urea (0.4 mL) to 0.9 mL of nanopure water to denature then dilute plasma samples. Following 25 minutes of stirring, each sample passed through a 30 mg Oasis® HLB solid-phase micro-extraction cartridge. We rinsed vials and cartridges with nanopure water 3 times. Analytes were eluted from the cartridges using 2-1 mL volumes of dichloromethane (DCM) and then dried using a weak flow of nitrogen gas. Internal standard compounds were added to vials and then dried again to monitor Gas Chromatograph (GC) performance. Lastly, analytes were

reconstituted with hexane, for a final volume of 0.1 mL, and quantified using an Agilent 7890 GC with an Electron Capture Device. We configured the GC with the quantitation techniques described in the Environmental Protection Agency method 8081 and 8082, with a split injection and dual columns. We developed individual analyte calibration curves for quantification and calculated detection limits. A calibration solution was also run with every set of extracted plasma samples to monitor for sample carry over, calibration performance and possible GC contaminations. Spike recoveries in each set of extracted plasma samples were required to average between 70 to 130% of the nominal concentration of analytes to meet quality assurance protocol standards for further statistical analyses. If the spikes of a set did not meet these recoveries, we repeated the extraction and cleanup method.

We only performed statistical analyses on PCB congeners that were present above the method detection limit (MDL) in greater than 50% of nestling plasma samples. PCB concentration values are presented as µg/kg wet weight or percent composition µg/kg wet weight. Each sample was measured for twenty PCB congeners including PCB 8 (2,4'-dichlorobiphenyl), PCB 18 (2,2',5-trichlorobiphenyl), PCB 28 (2,4,4'-trichlorobiphenyl), PCB 44 (2,2',3,5'-tetrachlorobiphenyl), PCB 52 (2,2',5,5'-tetrachlorobiphenyl), PCB-66 (2,3',4,4'-tetrachlorobiphenyl), PCB 101 (2,2',4,5,5'-pentachlorobiphenyl), PCB 105 (2,3,3',4,4'-pentachlorobiphenyl), PCB 110 (2,3,3',4',6-pentachlorobiphenyl), 118 (2,3',4,4',5-pentachlorobiphenyl), 128 (2,2',3,3',4,4'-hexachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl), 156 (2,3,3',4,4',5-hexachlorobiphenyl), 170 (2,2',3,3',4,4',5-heptachlorobiphenyl), 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl), 187

(2,2',3,4',5,5',6-heptachlorobiphenyl), 195 (2,2',3,3',4,4',5,6-octachlorobiphenyl), 206 (2,2',3,3',4,4',5,5',6-nonachlorobiphenyl), 209 (decachlorobiphenyl). PCB 77 (3,3',4,4'-tetrachlorobiphenyl) and PCB 126 (3,3',4,4',5-pentachlorobiphenyl) were also measured from 2005 to 2014 but only used for TEQ analyses.

Statistical Methods

Michigan was initially divided into 2 spatial regions: Inland (IN) and Great Lakes (GL). The IN region included all breeding areas located > 8km from a Great Lakes shoreline. The GL region included any breeding area < 8 km from a Great Lakes shoreline or along an anadromous river open to Great Lakes fish runs. The Kaplan-Meier method was used to report the potential range of the mean for congeners when non-detects were present in less than half of the samples for IN and GL spatial regions. For further spatial analyses, the IN spatial region was divided into four individual watersheds: 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). Lake Erie Inland was not included due to small sample size. The GL spatial region was also divided into four individual watersheds: Lake Huron Great Lake (LH-GL), Lake Michigan Great Lake (LM-GL), Lake Superior Great Lake (LS-GL), and Lake Erie Great Lake (LE-GL; Figure 2.1).

All analyses were conducted in *R* (R Development Core Team 2015).

Statistical comparisons among spatial regions in patterns of PCB compositions were made using analysis of similarity (ANOSIM), a multivariate analog of analysis of variance. ANOSIM is built on a nonparametric permutation procedure and applied to the rank similarity matrix underlying the ordination of samples (Clarke 1999). The

test statistic R ranges from -1 to $+1$, with zero meaning the distribution of patterns is as similar among groups as within groups and $+1$ meaning there are very clear differences in patterns among the groups being tested, in this case regions. An R value of ≥ 0.4 indicated some support for pattern differences and an R value of < 0.3 indicated little difference (Custer et al. 2010). Concentrations below the limits of quantification by the GC were replaced with half of the detection limit found for each individual congener (Leith et al. 2010). Due to the large number of decimal values resulting in negative values following a log transformation, contaminant data were $\log(x+1)$ transformed prior to ANOSIM analyses. Outliers were removed from the dataset using a Walsh's test with an α level of 0.05.

When spatial differences were detected, the similarity percentage (SIMPER) subroutine was performed to calculate the contribution to the difference for individual congeners. Bray-Curtis resemblance measures were used for both ANOSIM and SIMPER analyses. The $\log(x+1)$ concentration data were standardized (converted to percent of sum PCB concentration by sample) for compositional analysis to remove the effect of concentration differences for assessment of congener patterns (Custer et al. 2010). Compositional analyses also included Principal Component Analysis (PCA) on the proportions of individual PCB congeners to provide more insight into species congener patterns for spatial regions. PCA analyses were only conducted on specific congeners where the percent frequency of samples that were detected above the MDL was greater than 50%. Data for these congeners were also divided into three time periods: One (1999–2005), Two (2006–2009), and Three (2010–2014) to visualize changes in percent contribution for the four GL spatial regions.

Statistical time trend lines were performed on original concentrations from 1999-2014 on PCB 138 and PCB 153 for the IN spatial region, and PCB 52, PCB 66, PCB 101, PCB 105, PCB 118, PCB 138, PCB 153, PCB-170, and PCB 180 for the GL spatial region because these congeners were detected in 50% or greater of samples for these spatial regions. Normality was not achieved for parametric modeling due to the large percentage of censored values. The natural logs were taken of the concentrations to achieve a linear pattern before computing Akritas-Theil-Sen slope estimates for robust nonparametric linear regression accounting for censored (values below the MDL) data, using the Turnbull estimate of intercept. Kendall's *tau* correlation coefficient for singly censored data was used, along with the p-value for testing of significance (Helsel 2011; Lee 2013). A significance level of 0.05 determined whether the Kendalls's *tau* correlation coefficient was statistically significant. A two-sided p-value that was less than or equal to 0.05 indicated that there was a statistically significant correlation between time and concentration. P-values greater than 0.05 indicated that there was no statistically significant correlation between time and concentration. If a significant correlation existed, the sign of the slope indicated whether there was an increasing or decreasing trend (Bartholomay et al. 2012). Non-linear trends and 95% confidence regions of congeners detected above the MDL in 50% of the samples or greater in combined IN and GL spatial regions (LE-GL, LH-and LM-GL, and LS-GL) were plotted with a smoothed conditional mean using the predict method (Wickham 2009). To clarify, due to the

World Health Organization (WHO) toxic equivalency factors (TEFs) for birds were used to calculate toxic equivalency values (TEQs) (van den Berg et al. 1998).

These TEF values have slightly changed since 1998 for mammals but have not changed for avian species (van den Berg et al. 2006). The three mono-*ortho*, dioxin-like congeners calculated and their corresponding TEFs are PCB 105, 118, 156 and 0.0001, 0.00001, 0.0001, respectively. Two non-ortho, dioxin-like congener are PCB 77 and 126 with a TEF of 0.05 and 0.1, respectively. Contaminant concentrations for PCB 77 and 126 in bald eagle plasma was only available from 2005 to 2014 so they were only included in TEQ analyses. The concentrations of each congener were multiplied by the TEF coefficient for that congener. All values were then summed (Custer et al. 2010). The Peto-Prentice version of the generalized Wilcoxon (Gehan) test, accounting for censored values, was used to determine whether the distribution of TEQs differed significantly among spatial regions (Lee 2013). Due to the lack of ideal multiple comparison tests available for censored data, a series of two-group score tests between each spatial region were performed when the Gehan test resulted in a significant value. If the *p*-value from the two-group score tests was less than the Bonferroni-adjusted individual comparison level calculated as:

$$\text{individual error rate} = \frac{\alpha}{g}$$

where α is the overall error rate (0.05) and *g* is the number of comparisons to be made (8 spatial regions), the two spatial regions were declared to have different empirical cumulative distribution functions (Helsel 2011).

To compare differences in the treatment of the large number of samples measured below the MDL, we performed three different analyses to calculate TEQs. The first method, abbreviated as NND (on non-detects) did not include samples found to be below the MDL to multiply by TEFs. When all values were below the MDL for

every congener in a sample, a zero was used to sum the TEQs. The second method, abbreviated as HND (half non-detects) used a value of $\frac{1}{2} * \text{MDL}$ of each congener to multiply by the TEF. For the third method, abbreviated as RND (random non-detect), we generated a random value between zero and the MDL of each congener to multiply by the TEF. Random numbers were generated using the Excel (Microsoft) iterative solver function. TEQs are reported in ng/kg.

Results

Twenty PCB congeners were measured in 1,172 bald eagle nestling plasma samples from 1999 to 2014. Those congeners included PCB-8, 18, 28, 52, 44, 66, 101, 110, 118, 153, 105, 138, 187, 128, 156, 180, 170, 195, 206, and 209. PCB-77 and 126 were also measured in plasma samples from 2005 to 2014. Congeners which were detected above the MDL in 50% of the samples or greater were PCB 138 and 153 for the IN spatial region. Therefore, these were the only congeners used for further analyses for the IN spatial region (Table 2.1). Congeners detected above the MDL in 50% of the samples or greater were PCB 52, 66, 101, 105, 118, 138, 153, 170, and 180 for the GL spatial region. Again, these were the only congeners used for further analyses for the GL spatial region.

Spatial Patterns

The pattern of PCB congener concentrations as a whole differed between IN and GL spatial regions (ANOSIM $R = 0.398$, $p < 0.001$). The major congeners (contribution of 3% or greater) that differentiated the GL from IN spatial regions in order of decreasing percent contribution of differences were PCB 8 (12%), 138 (9%),

153 (8%), 18 (8%), 52 (8%), 180 (4%), 118 (3%). There were no differences among IN spatial regions (ANOSIM $R = -0.00964$, $p = 0.881$), so all IN regions were combined for further analyses. There were also no differences among GL spatial regions (ANOSIM $R = 0.1601$ $p < 0.001$). We performed a Principal Component Analysis (PCA) on only congeners in which less than 50% of samples were below the detection limit in the GL PCA (Figure 2.2). Based on visual differences observed, pairwise ANOSIM comparisons were made between GL spatial regions, with LS-GL differing from LE-GL (ANOSIM $R = 0.5304$, $p < 0.001$; Table 2.2). LS-GL also showed slight differences between LH-GL and LM-GL (ANOSIM $R = 0.2797$ and $R = 0.2149$, respectively; Table 2.2). The major congeners (contribution of 3% or greater) that differentiated LS-GL from LE-GL spatial regions in order of decreasing percent contribution of differences were PCB 138 (11%), 153 (10%), 52 (7%), 66 (6%), 180 (5%), 105 (4%), and 101 (4%). These results are further observed in the graphs of percent congener composition (Figure 2.8). Lower-chlorinated congeners, such as PCB 52 and 66, comprise LE-GL to a greater degree than LS-GL in which higher chlorinated congeners, such as PCB 138 and 153, are more abundant.

Temporal Trends

Concentrations of PCB-138 and 153 were the most frequently-detected congeners for both IN and GL spatial regions. These congeners also were detected at the greatest concentrations in IN and GL spatial regions (Table 2.1). Akritas-Theil-Sen lines detected no significant trends for PCB-138 in all spatial regions (Table 2.3; Figure 2.4). The percentage plots indicate an increase in the percent contribution of PCB-138 to total PCB concentrations around 2003 (Figure 2.5). This may partially

explain the lack a detected negative trend. A significant negative Akritas-Theil-Sen line trend was detected for PCB 153 (Table 2.3).

Two congeners, PCB 52, and PCB 66 showed significant increasing Akritas-Theil-Sen line trend for LH-GL, LM-GL, and LS-GL spatial regions (Table 2.3). The annual percent change for LS-GL was greater than all other congeners and regions due to the greater number of MDL values from 1999 until 2004. These congeners began to be detected more frequently from 2005 to 2014. Because the concentrations detected are somewhat negligible, the large annual percent change is not reflected in the plot lines (Table 2.4). Increasing trends of PCB 52 and 66 are most clearly observed for LH-GL in the percent composition graphs (Figure 2.8). The sample size of the spatial region LE-GL was generally small (<24 samples) for each congener, increasing variation of the means and subsequent 95% confidence intervals (Figures 2.4 and 2.7). Despite this, the percent of values detected was often greater than 90% and the means were greater than other spatial regions for PCB 52, 66, 101, 170, and 180 (Figure 2.4). These larger detections and means however, did not result in increasing trends. LE-GL showed no significant Akritas-Theil-Sen line trends except for PCB 101, 118, and 153, where they were decreasing. Akritas-Theil-Sen line trends should be considered with caution, however. Although Akritas-Theil-Sen trends do not make assumptions about the distribution of the residuals of the data, computing a single slope assumes that the data follow a linear pattern. Despite natural log transformations, concentration data remained non-linear. Unfortunately, Akritas-Theil-Sen line trends are the only available option for highly censored (up to 50% of

samples <MDL), nonparametric data (Helsel 2011). A polynomial regression may better explain the non-linear trends in data.

Toxic Equivalents

TEQs were greatest in GL spatial regions, mainly LE-GL, LH-GL, and LM-GL (Tables 2.4, 2.5, and 2.6). The RND method, which substituted a randomly generated number between 0 and the MDL, estimated the lowest mean of LE-GL (298.50 ng/kg; Table 2.6). This was followed by the NND method (328.23 ng/kg; Table 2.4), which substituted zeros to sum TEQs when all congener values were below the MDL, and the HND method (342.25 ng/kg; Table 2.5) which substituted values with $\frac{1}{2} * \text{MDL}$. TEQ means ranged from 147.35 to 195.72 ng/kg in the LH-GL spatial region. TEQ means ranged from 114.99 to 154.63 ng/kg in the LM-GL spatial region. TEQs means in LS-GL were greater than IN spatial regions, but similar in that it had a greater number (almost 50%) of samples with concentrations below the MDL. TEQs in all IN spatial regions were significantly less than GL spatial regions. The distribution of LS-IN was significantly different from LM-IN-UP, but not from LM-IN-LP. This is likely due to the greater number of values below the MDL in both LS-IN and LM-IN-LP regions. Aside from LE-GL, HND and RND methods estimated means of all other spatial regions similarly (Table 2.5, Table 2.6). The NND estimated means to be 20-50% less than methods one and two, with a greater disparity as the number of values below the MDL increased.

Discussion

The pattern of all PCB congeners differed between IN and GL spatial regions. Only two congeners, PCB-138 and 153, were detected in greater than 50% of samples for IN regions. Mean concentrations of these congeners were greater in GL spatial regions than IN spatial regions. Similar to present findings, Bowerman et al. (2003) noted significantly greater geometric mean concentrations of total PCBs in nestling plasma from GL breeding areas between 1987 and 1992. These greater concentrations were also inversely correlated with productivity, or total number of fledged young per occupied nest, in GL breeding areas. Only congeners PCB 138 and 153 were detected in 50% or greater of the samples for the IN spatial regions. These were also the most frequently-detected congeners in the GL spatial region. Structurally, PCB 138 and 153 are similar in that they are hexa-chlorinated congeners (Figure 2.3d). These highly chlorinated congeners have been shown to be dominant in multiple avian studies due to higher octanol/ water partition coefficients (7.441–7.751), or increased hydrophobicity, resulting in decreased elimination rates and biomagnification (Eisler and Belisle 1996). More than 50% of total PCB concentrations in eggs of the yellow-legged herring gull (*Larus cachinnans*) were comprised of PCBs 138, 153, and 180 (Focardi et al. 1988a), as well as 13.5% of PCB 153 in infertile imperial eagle (*Aquila heliaca adalberti*) eggs (Hernández et al. 1989). PCB 153, was the most abundant congener, followed by PCB 138 in herring gull (*Larus argentatus*) eggs collected from Big Sister Island in Green Bay, Lake Michigan and Scotch Bonnet Island in Lake Ontario from 1971 to 1982 (Hebert et al. 1999). Inputs of these congeners seems to be rather constant, at minimal concentrations, for the IN spatial region, followed by

an increase and peak around 2006 or 2007. This trend is slightly different for GL spatial regions, with a delayed increase, and subsequent peak by three to four years. The temporal trends of PCB 138 and 153 between IN and GL spatial regions provide evidence of input and loss fluctuations between spatial regions. PCB 138 seems to be more persistent however, as no overall trend was detected for any spatial region and the percent of total PCBs is increasing in LE-GL, LM-GL, and LH-GL (Figure 2.5), in comparison to PCB 153 which was shown to be decreasing in all spatial regions (Table 2.3; Figure 2.4). The annual percentage changes we observed (-5.8 to -8.5) were comparable to concentrations of PCB 153 in northern pike (*Esox lucius*) and Arctic char (*Salvelinus alpinus*) in Sweden, which were decreasing by 3 to 8% per year (Table 2.4) (Nyberg et al. 2014).

The greater percentage of hexa-chlorinated congeners in remote regions such as LS-GL is likely a result of long-range atmospheric transport. It has been shown that PCB concentrations are ubiquitous in the North American environment and appear to be driven predominantly by their vapor pressure and thus, volatilized for transport following rises in temperature (Hoff et al. 1992). Long-range atmospheric transport following volatilization is a major pathway for non-point deposition and distribution of PCBs (Gioia et al. 2013; Scheringer 2009). In addition, removal of PCBs from air by snowfall is a major factor that potentially reduces their long-range transport. After deposition via snowfall, relatively lighter and more volatile PCBs revolatilize. Heavier, or higher chlorinated PCBs, are bound to particles in the snow-pack and are released to soils following snowmelt or into Great Lakes-bound waterways, depending on solubility properties (Scheringer 2009). Less soluble

congeners will bind to sediments, where they will persist due to the cooler air and water temperatures (Grimalt et al. 2004; Iwata et al. 1995). In addition, deposition rates are greater than volatilization in areas of cooler climates and long winters (Gregor and Gummer 1989). This lends support to the hypothesis of the Upper Midwest being a potential sink for historical depositions of PCBs (Pittman et al. 2015). Meijer et al. (2003) reported amounts of PCB congeners present in soils at three different longitudinal regions (Region I, 90°S–30°N; Region II, 30°N–60°N; and Region III, 60°N–90°N), with the main sources of PCBs located in Region II. Lighter PCB congeners were found to be more broadly distributed than heavier congeners. Substantial amounts (hundreds of tonnes) of heavy PCB congeners however, also transported from sources. Further analyses of PCB molecular weight profile increases from eastern to western Lake Superior may provide insight into the link between long-range transport and atmospheric deposition in the Midwest (Anthony et al. 2007).

Greater detection frequencies and mean concentrations were observed in LE-GL for individual congeners despite the least number of samples collected. There were however, either no trends, or decreasing trends detected for all individual congeners in the LE-GL spatial region. These results are consistent with a study conducted by the Integrated Atmospheric Deposition Network (IADN), who found that half-lives for many congeners and total PCBs at their sampling site on Lake Erie between 1990 and 2003 were about 20 years. The authors suggested that the slower rate of decrease in atmospheric concentrations possibly indicate that Lake Erie is now approaching steady state after a rapid decline from 1975 to 1995 (Buehler et al. 2004;

Sun et al. 2007). These longer half-lives were also observed in lake trout (*Salvelinus namaycush*) sampled from Lake Erie compared to other Great Lakes (Hickey et al. 2006).

Although PCB 52 and 66 did not greatly contribute to total congener composition for LH-GL, LM-GL, or LS-GL spatial regions, these were the only congeners with increasing trends. This finding is particularly interesting as these are less stable, lower (tetra- and penta-) chlorinated congeners (Figure 2.3a), that are expected to occur mainly near local urban or industrial input sources (Nyberg et al. 2014). These increasing trends may be a result of the linear nature of the Akritas-Theil-Sen line statistic and should be considered with caution. A polynomial regression may have accounted for the large increase beginning in 2002, and peaking from 2008 to 2009. The percent composition in LH-GL and LM-GL for PCB 66 in however, is sustained even after the 2008 and 2009 peak (Figure 2.7). Sun et al. (2007) found a strong positive correlation between total PCB concentrations and human population within a 25 km radius of their urban sampling site of Chicago. The authors suggested Chicago as a source of PCBs for Lake Michigan as higher concentrations have been measured in air and precipitation (Offenberg and Baker 1997; Sun et al. 2006; Tasdemir et al. 2004). Specifically, Hsu et al. (2003) identified sludge drying beds, a large landfill, and a transformer storage yard as sources of PCBs in Chicago.

A definitive increase in the percent composition of these lighter congeners is most clearly observed for LH-GL (Figure 2.8). Local PCB sources for LH-GL may be contaminated sites located within the Saginaw Bay Area of Concern (SGB AOC).

The SGB AOC, created under the Great Lake's Water Quality Agreement, is comprised of the length of the Saginaw River and all of Saginaw Bay. The Saginaw River and Bay were designated as an AOC due to contaminated sediments, fish consumption advisories, degraded fisheries, and loss of significant recreational values, primarily from nonpoint source discharges, combined sewer overflows, sanitary sewer overflows, and industrial sources of polychlorinated biphenyls (Selzer and Bureau 2008). Dredging projects were implemented as restoration efforts in the SGB AOC. The first major project was completed in July 2001. 342,433 cubic yards (578,712 tonnes) of contaminated sediments were transported by barge to a confined disposal facility just outside the mouth of the Saginaw River (Service 2015).

Dredging activities also occurred in the lower Saginaw River and Saginaw Bay in 2007 (Selzer and Bureau 2008). Dredged sediments may therefore, act as a source of lighter chlorinated congeners for the LH-GL watershed. Increases in the percentage of PCB 52 and 66 can also be partially attributed to the greater vapor pressures (0.0105 Pa and 0.0565 Pa, respectively) and lower octanol/ air partition coefficients ($\log K_{oa}$: 8.470 and 9.020, respectively), resulting in greater volatilization rates and mobility from local sources due to weather-related changes such as temperature (ChemSpider 2015).

Increases in concentrations of all congeners and TEQs (Figures 2.4, 2.5, and 2.7) in the early 2000's, which peaked between 2008–2010, may likely be due to large-scale environmental factors. Higher southern U.S. temperatures in previous years may have led to volatilization, northern atmospheric transport, and deposition. This may have also been enhanced by winters with greater snowfall. Further

modeling is needed however, to assess correlations of temperature and snowfall on PCB concentrations in the Great Lakes. Another possible explanation of the observed trends is a shift in aquatic prey in the Great Lakes, resulting in a change of trophic-level exposure. The most recent structural food web change occurred around 2003. Before this, the food webs of Lakes Michigan and Huron were dominated by invasive prey fish, such as alewives (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*), and introduced Chinook salmon (*Oncorhynchus tshawytscha*). Salmon populations were largely dependent on annual stocking (Whelan and Johnson 2005). The tertiary-level piscivores in Lakes Erie and Superior however, were two native species, walleye (*Sander vitreus*) and lake trout (*Salvelinus namaycush*), which were regulated by natural recruitment (Hartman and Margraf 1992; Kitchell et al. 2000). Major changes occurred in the Great Lakes, particularly Lake Huron, following the invasion of zebra (*Dreissena polymorpha*) and quagga mussels (*Dreissena bugensis*), which altered lower-trophic energy cycling and eventually led to the collapse of alewives in 2003. The collapse of alewives led to subsequent declines in Chinook salmon abundance (He et al. 2015). This resulted in an increase in natural recruitment, age, and size of lake trout (He et al. 2012). Rapid increases in Walleye recruitment and abundance also occurred in the Saginaw Bay (Fielder et al. 2007). These shifts may give insight into the trends of congener concentrations during our study period. It may also explain the changes in percent composition of congeners observed in individual lakes, such as the increase of lighter congeners in Lake Huron.

Mono-*ortho* and non-*ortho* coplanar PCBs (Figure 2.3b and c) have been shown to largely contribute to TCDD-like toxicity in fish-eating birds (Bosveld et al.

1995; Senthil Kumar et al. 2002; van den Berg et al. 1994). These adverse effects are caused by Ah-receptor-mediated responses and can be measured by the induction of the hepatic cytochrome P4501A (CYP1A) cross-reactive protein (Elliott et al. 1996a; Hoffman et al. 1987; Rattner et al. 1994; Sanderson et al. 1994). The no-observed-adverse-effect-level (NOAEL) and the lowest-observed-adverse-effect-level (LOAEL) bald eagle eggs is 100 ng/kg and 210 ng/kg, respectively (Elliott et al. 1996a; Su et al. 2014b). Although TEQs were greatest in the GL spatial regions, particularly LE-GL, determining the level of risk in these areas is difficult as studies reporting concentrations of dioxin-like compounds in avian nestling plasma and subsequent effects are limited. A number of studies however, have examined the relationships between PCB exposure and thyroid hormone status in both children and adults. The results suggest that PCBs can induce thyroid toxicity as well as a variety of changes in thyroid hormone levels. Nagayama et al. (1998) found a significant negative correlation between TEQ intake (14–68 ng/kg) in breast milk and thyroxine levels in blood plasma of 36 breast-fed infants the second week after birth. Depletion of circulating thyroid hormones in the fetus or neonate due to PCB exposure *in utero* and/or during early development (e.g., through breast milk) can result in a hypothyroid state during development. Given this, elevated exposure to PCB TEQs could alter the status of both animal and human thyroid hormones, leading to hypothyroidism and associated neurodevelopmental disorders and deficits (Health and Services 2000).

Tables

Table 2.1 Concentrations ($\mu\text{g/kg ww}$) of polychlorinated biphenyl (PCB) congeners in bald eagle plasma (*Haliaeetus leucocephalus*) in Michigan (USA) from 1999–2014.

| Congener | IN (n = 598) ¹ | GL (n = 557) ¹ |
|----------|--|--|
| | Geometric mean ² extremes n detected MDL | Geometric mean ² extremes n detected MDL |
| 8 | — <MDL–19.55 66 2.01 | — <MDL–26.16 63 2.01 |
| 18 | — <MDL–2.60 4 1.59 | — <MDL–32.25 27 1.59 |
| 28 | — <MDL–7.30 8 0.18 | — <MDL–60.80 51 0.18 |
| 44 | — <MDL–5.39 13 0.001 | — <MDL–42.16 139 0.001 |
| 52 | — <MDL–12.99 116 0.001 | 2.31 <MDL–64.06 285 0.001 |
| 66 | — <MDL–33.61 39 0.001 | 3.21 <MDL–61.25 339 0.001 |
| 77 | — <MDL–22.39 4 2.43 | — <MDL–15.59 92 2.43 |
| 101 | — <MDL–18.72 42 0.001 | 2.44 <MDL–31.10 301 0.001 |
| 105 | — <MDL–40.44 86 0.07 | 1.95 <MDL–17.40 271 0.07 |
| 110 | — <MDL–12.47 91 0.001 | — <MDL–31.00 189 0.001 |

Table 2.1 Continued

| | | |
|-----|-------------|-------------|
| 118 | – | 3.97 |
| | <MDL–41.40 | <MDL–37.12 |
| | 104 | 349 |
| | 0.001 | 0.001 |
| 126 | – | – |
| | <MDL–33.49 | <MDL–17.83 |
| | 19 | 79 |
| | 2.00 | 2.00 |
| 128 | – | – |
| | <MDL–35.31 | <MDL–14.71 |
| | 48 | 220 |
| | 0.001 | 0.001 |
| 138 | 2.81 | 10.20 |
| | <MDL–76.38 | <MDL–87.99 |
| | 307 | 497 |
| | 0.001 | 0.001 |
| 153 | 3.19 | 9.97 |
| | <MDL–128.06 | <MDL–100.33 |
| | 298 | 515 |
| | 0.001 | 0.001 |
| 156 | – | – |
| | <MDL–20.94 | <MDL–10.15 |
| | 24 | 89 |
| | 0.001 | 0.001 |
| 170 | – | 1.85 |
| | <MDL–65.12 | <MDL–30.00 |
| | 89 | 279 |
| | 0.001 | 0.001 |
| 180 | – | 5.59 |
| | <MDL–127.69 | <MDL–57.64 |
| | 202 | 445 |
| | 0.001 | 0.001 |
| 187 | – | – |
| | <MDL–12.56 | <MDL–28.93 |
| | 92 | 197 |
| | 0.001 | 0.001 |
| 195 | – | – |
| | <MDL–4.49 | <MDL–4.34 |
| | 14 | 58 |
| | 0.001 | 0.001 |
| 206 | – | – |
| | <MDL–15.70 | <MDL–13.44 |
| | 22 | 91 |
| | 0.001 | 0.001 |
| 209 | – | – |
| | <MDL–1.40 | <MDL–15.40 |
| | 6 | |
| | 0.001 | 0.001 |

¹ – IN (n = 311) and GL (n = 303) for PCB-77 and 126

² – No mean calculated, as more than half of the samples were below the method detection limit (MDL); extremes are defined as the minimum and maximum values in the dataset.

Table 2.2 Analysis of Similarity (ANOSIM) pairwise comparisons between concentrations of polychlorinated biphenyl (PCB) congeners in bald eagle plasma (*Haliaeetus leucocephalus*) in Michigan (USA) Great Lakes spatial regions from 1999–2014.

| Lake Comparison | ANOSIM R | P value |
|-----------------------------|----------|---------|
| Lake Erie–Lake Huron | -0.03899 | 0.756 |
| Lake Erie–Lake Michigan | -0.03087 | 0.752 |
| Lake Erie–Lake Superior | 0.5304* | 0.001 |
| Lake Huron–Lake Michigan | 0.01839 | 0.012 |
| Lake Huron–Lake Superior | 0.2797 | 0.001 |
| Lake Michigan–Lake Superior | 0.2149 | 0.001 |

* Differences in distribution patterns between spatial regions.

Table 2.3 Annual percentage change, Kendall's tau, p value (significant at <0.05), and trend of regression line for polychlorinated biphenyl (PCB) congeners 52, 66, 101, 105, 118, 138, 153, 170, 180 calculated using the Akritas-Theil-Sen method in bald eagle (*Haliaeetus leucocephalus*) plasma samples collected in Michigan (USA) from 1999-2014. All Inland (IN) spatial regions were combined for PCB congeners 138 and 153. The Great Lake (GL) spatial regions of Lake Erie and Lake Superior were analyzed and reported separately for all congeners. The GL spatial regions of Lake Huron and Lake Michigan were analyzed and reported together for all congeners.

| Congener | Spatial Region | Mean % of Total PCBs | Annual % change ¹ | Kendall's <i>tau</i> | <i>p</i> value | Trend |
|----------------|----------------------------|----------------------------|---------------------------------|-------------------------|----------------|----------|
| PCB-52 | Lake Erie GL | 11 | -6.2 | -0.15 | 0.324 | no trend |
| | Lake Huron and Michigan GL | 4.5 | 20.6 | 0.222 | P<0.001* | + |
| | Lake Superior GL | 2 | 246.1 | 0.164 | P<0.001* | + |
| PCB-66 | Lake Erie GL | 9.8 | -4.1 | -0.13 | 0.395 | no trend |
| | Lake Huron and Michigan GL | 6.7 | 9.4 | 0.205 | P<0.001* | + |
| | Lake Superior GL | 2.5 | 134.9 | 0.076 | 0.05* | + |
| PCB-101 | Lake Erie GL | 6.9 | -6.2 | -0.308 | 0.04* | — |
| | Lake Huron and Michigan GL | 5.3 | -3.4 | -0.071 | 0.031* | — |
| | Lake Superior GL | 1 | -64.1 | -0.114 | 0.005* | — |
| PCB-105 | Lake Erie GL | 2.2 | -1.1 | -0.015 | 0.932 | no trend |
| | Lake Huron and Michigan GL | 4 | 0.9 | 0.016 | 0.619 | no trend |
| | Lake Superior GL | 2.7 | -22.2 | -0.066 | 0.126 | no trend |
| PCB-118 | Lake Erie GL | 4.2 | -32.3 | -0.332 | 0.023* | — |
| | Lake Huron and Michigan GL | 8.1 | -19.0 | -0.344 | 0* | — |
| | Lake Superior GL | 6 | -53.6 | -0.185 | P<0.001* | — |
| PCB-138 | IN | 17.67 | -0.9 | -0.007 | 0.762 | no trend |
| | Lake Erie GL | 12 | -2.5 | -0.126 | 0.407 | no trend |
| | Lake Huron and Michigan GL | 19.3 | -2.3 | -0.101 | 0.318 | no trend |
| | Lake Superior GL | 22.3 | -2.7 | -0.056 | 0.318 | no trend |
| PCB-153 | IN | 20 | -8.5 | -0.06 | 0.015* | — |
| | Lake Erie GL | 12.9 | -5.8 | -0.431 | 0.004* | — |
| | Lake Huron and Michigan GL | 18.3 | -6.9 | -0.289 | 0* | — |
| | Lake Superior GL | 23.2 | -7.6 | -0.155 | 0.006* | — |
| PCB-170 | Lake Erie GL | 4.5 | -0.7 | -0.032 | 0.852 | no trend |
| | Lake Huron and Michigan GL | 3.2 | -11.7 | -0.115 | P<0.001* | — |
| | Lake Superior GL | 4.4 | -46.8 | -0.083 | 0.076 | no trend |
| PCB-180 | Lake Erie GL | 10.4 | -4.2 | -0.217 | 0.152 | no trend |
| | Lake Huron and Michigan GL | 10 | -5.8 | -0.208 | P<0.001* | — |
| | Lake Superior GL | 12.8 | -34.0 | -0.169 | 0.002* | — |

¹Percent change in *Y* per year = $(e^{b1} - 1) \times 100$; e^{b1} = Akritas-Theil-Sen slope estimate.

*Significant correlation between time and concentration.

Table 2.4 Concentrations (ng/kg) of summed toxic equivalents (TEQs) of polychlorinated biphenyl (PCB) congeners 77, 105, 118, 126, and 156 in bald eagles (*Haliaeetus leucocephalus*) from Michigan (USA) Inland (IN) and Great Lakes (GL) spatial regions, 1999–2014. Samples were given a summed value of zero when measured to be below the minimum detection limit (MDL) for all congeners.

| Spatial Region ² | <i>n</i> / <i>n</i> <MDL | Mean ¹ | Standard Deviation | Comparison ² | Chi-Square | p value* |
|-----------------------------|--------------------------|----------------------|--------------------|-------------------------|------------|----------|
| Lake Erie GL | 23/ 3 | 328.23 ^a | 480.87 | Lake Huron GL | 1.5 | 0.226 |
| | | | | Lake Michigan GL | 3.2 | 0.0753 |
| | | | | Lake Superior GL | 31.9 | <0.001 |
| | | | | Lake Huron IN | 84.3 | 0 |
| | | | | Lake Michigan IN LP | 86.5 | 0 |
| | | | | Lake Michigan IN UP | 73.1 | 0 |
| | | | | Lake Superior IN | 87.9 | 0 |
| Lake Huron GL | 225/ 35 | 147.35 ^a | 270.70 | Lake Michigan GL | 2.1 | 0.152 |
| | | | | Lake Superior GL | 65.7 | <0.001 |
| | | | | Lake Huron IN | 181 | 0 |
| | | | | Lake Michigan IN LP | 125 | 0 |
| | | | | Lake Michigan IN UP | 130 | 0 |
| | | | | Lake Superior IN | 98.7 | 0 |
| Lake Michigan GL | 166/ 34 | 114.99 ^a | 333.91 | Lake Superior GL | 43.7 | <0.001 |
| | | | | Lake Huron IN | 138 | 0 |
| | | | | Lake Michigan IN LP | 104 | 0 |
| | | | | Lake Michigan IN UP | 100 | 0 |
| | | | | Lake Superior IN | 85.7 | 0 |
| Lake Superior GL | 143/ 71 | 65.37 ^b | 274.08 | Lake Huron IN | 22.1 | <0.001 |
| | | | | Lake Michigan IN LP | 26.3 | <0.001 |
| | | | | Lake Michigan IN UP | 10.3 | <0.001 |
| | | | | Lake Superior IN | 32.3 | <0.001 |
| Lake Huron IN | 239/ 179 | 41.68 ^c | 262.95 | Lake Michigan IN LP | 2.1 | 0.15 |
| | | | | Lake Michigan IN UP | 1.8 | 0.178 |
| | | | | Lake Superior IN | 7.9 | 0.00493 |
| Lake Michigan IN LP | 124/ 101 | 10.80 ^{c d} | 76.24 | Lake Michigan IN UP | 6.3 | 0.0122 |
| | | | | Lake Superior IN | 2.7 | 0.0998 |
| Lake Michigan IN UP | 160/ 107 | 5.27 ^c | 38.67 | Lake Superior IN | 13.7 | <0.001 |
| Lake Superior IN | 78/ 70 | 2.58 ^d | 24.13 | | | |

¹ Computed using the Kaplan–Meier method; different superscript letters indicate significant differences of TEQ distribution among spatial regions (generalized Wilcoxon with Bonferroni-adjusted individual comparison).

² Upper Peninsula and Lower Peninsula are abbreviated as UP and LP, respectively.

Table 2.5 Concentrations (ng/kg) of summed toxic equivalents (TEQs) of polychlorinated biphenyl (PCB) congeners 77, 105, 118, 126, and 156 in bald eagles (*Haliaeetus leucocephalus*) from Michigan (USA) Inland (IN) and Great Lakes (GL) spatial regions, 1999–2014. Samples measured to be below the minimum detection limit (MDL) were assigned a value of ½ * MDL for individual congeners then multiplied by corresponding toxic equivalency factors.

| Spatial Region ² | n / n<MDL | Mean ¹ | Standard Deviation | Comparison ² | Chi-Square | p value* |
|-----------------------------|-----------|----------------------|--------------------|-------------------------|------------|----------|
| Lake Erie GL | 23/ 6 | 342.25 ^a | 480.89 | Lake Huron GL | 0.5 | 0.488 |
| | | | | Lake Michigan GL | 1.1 | 0.284 |
| | | | | Lake Superior GL | 29.3 | <0.001 |
| | | | | Lake Huron IN | 68 | <0.001 |
| | | | | Lake Michigan IN LP | 65.6 | <0.001 |
| | | | | Lake Michigan IN UP | 69.9 | <0.001 |
| | | | | Lake Superior IN | 78 | 0 |
| Lake Huron GL | 225/ 38 | 188.64 ^a | 279.52 | Lake Michigan GL | 1.5 | 0.228 |
| | | | | Lake Superior GL | 65.8 | <0.001 |
| | | | | Lake Huron IN | 145 | 0 |
| | | | | Lake Michigan IN LP | 93.8 | 0 |
| | | | | Lake Michigan IN UP | 132 | 0 |
| | | | | Lake Superior IN | 84.7 | 0 |
| Lake Michigan GL | 166/ 36 | 154.63 ^a | 337.44 | Lake Superior GL | 48.8 | <0.001 |
| | | | | Lake Huron IN | 113 | 0 |
| | | | | Lake Michigan IN LP | 80.2 | 0 |
| | | | | Lake Michigan IN UP | 110 | 0 |
| | | | | Lake Superior IN | 77.2 | 0 |
| Lake Superior GL | 143/ 72 | 78.40 ^b | 285.66 | Lake Huron IN | 14.9 | <0.001 |
| | | | | Lake Michigan IN LP | 16.5 | <0.001 |
| | | | | Lake Michigan IN UP | 14.4 | <0.001 |
| | | | | Lake Superior IN | 31.4 | <0.001 |
| Lake Huron IN | 239/ 181 | 56.34 ^c | 272.45 | Lake Michigan IN LP | 1.5 | 0.217 |
| | | | | Lake Michigan IN UP | 0 | 0.899 |
| | | | | Lake Superior IN | 9.4 | 0.002 |
| Lake Michigan IN LP | 124/ 101 | 21.69 ^{c d} | 94.55 | Lake Michigan IN UP | 1.3 | 0.257 |
| | | | | Lake Superior IN | 4.3 | 0.038 |
| Lake Michigan IN UP | 160/ 108 | 14.48 ^c | 57.87 | Lake Superior IN | 10.7 | 0.001.7 |
| Lake Superior IN | 78/ 70 | 7.49 ^d | 41.19 | | | |

¹ Computed using the Kaplan–Meier method; different superscript letters indicate significant differences of TEQ distribution among spatial regions (generalized Wilcoxon with Bonferroni-adjusted individual comparison).

² Upper Peninsula and Lower Peninsula are abbreviated as UP and LP, respectively.

Table 2.6 Concentrations (ng/kg) of summed toxic equivalents (TEQs) of polychlorinated biphenyl (PCB) congeners 77, 105, 118, 126, and 156 in bald eagles (*Haliaeetus leucocephalus*) from Michigan (USA) Inland (IN) and Great Lakes (GL) spatial regions, 1999–2014. Samples measured to be below the minimum detection limit (MDL) were assigned a random value between 0 and the MDL for individual congeners then multiplied by corresponding toxic equivalency factors.

| Spatial Region ² | n / n<MDL | Mean ¹ | Standard Deviation | Comparison ² | Chi-Square | p value* |
|-----------------------------|-----------|----------------------|--------------------|-------------------------|------------|----------|
| Lake Erie GL | 23/ 6 | 298.50 ^a | 496.16 | Lake Huron GL | 0 | 0.859 |
| | | | | Lake Michigan GL | 0 | 0.928 |
| | | | | Lake Superior GL | 17.7 | <0.001 |
| | | | | Lake Huron IN | 42.8 | <0.001 |
| | | | | Lake Michigan IN LP | 43.8 | <0.001 |
| | | | | Lake Michigan IN UP | 49.5 | <0.001 |
| | | | | Lake Superior IN | 60.6 | <0.001 |
| Lake Huron GL | 225/ 38 | 195.72 ^a | 285.91 | Lake Michigan GL | 1.8 | 0.182 |
| | | | | Lake Superior GL | 64.7 | <0.001 |
| | | | | Lake Huron IN | 139 | 0 |
| | | | | Lake Michigan IN LP | 87.9 | 0 |
| | | | | Lake Michigan IN UP | 131 | 0 |
| | | | | Lake Superior IN | 80.7 | 0 |
| Lake Michigan GL | 166/ 36 | 151.59 ^a | 339.19 | Lake Superior GL | 47.6 | <0.001 |
| | | | | Lake Huron IN | 105 | 0 |
| | | | | Lake Michigan IN LP | 72.3 | 0 |
| | | | | Lake Michigan IN UP | 110 | 0 |
| | | | | Lake Superior IN | 71.6 | 0 |
| Lake Superior GL | 143/ 72 | 76.26 ^b | 284.85 | Lake Huron IN | 14.3 | <0.001 |
| | | | | Lake Michigan IN LP | 14.9 | <0.001 |
| | | | | Lake Michigan IN UP | 15 | <0.001 |
| | | | | Lake Superior IN | 30.1 | <0.001 |
| Lake Huron IN | 239/ 181 | 55.24 ^c | 275.71 | Lake Michigan IN LP | 1.1 | 0.296 |
| | | | | Lake Michigan IN UP | 0 | 0.998 |
| | | | | Lake Superior IN | 8.4 | 0.004 |
| Lake Michigan IN LP | 124/ 101 | 23.73 ^{c d} | 102.17 | Lake Michigan IN UP | 0.7 | 0.391 |
| | | | | Lake Superior IN | 4.2 | 0.0401 |
| Lake Michigan IN UP | 160/ 108 | 13.56 ^c | 57.51 | Lake Superior IN | 9.7 | 0.0018 |
| Lake Superior IN | 78/ 70 | 8.68 ^d | 46.92 | | | |

¹ Computed using the Kaplan–Meier method; different superscript letters indicate significant differences of TEQ distribution among spatial regions (generalized Wilcoxon with Bonferroni-adjusted individual comparison).

² Upper Peninsula and Lower Peninsula are abbreviated as UP and LP, respectively.

Figures

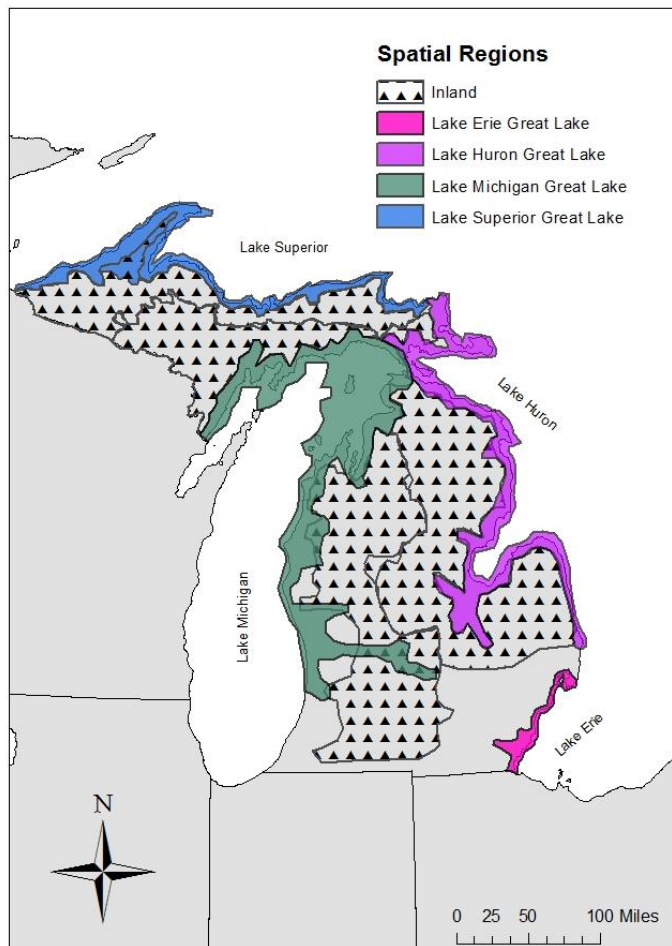


Figure 2.1 Spatial regions of Michigan (USA) where samples were collected from bald eagles (*Haliaeetus leucocephalus*) to measure concentrations of polychlorinated biphenyl (PCB) congeners from 1999–2014.

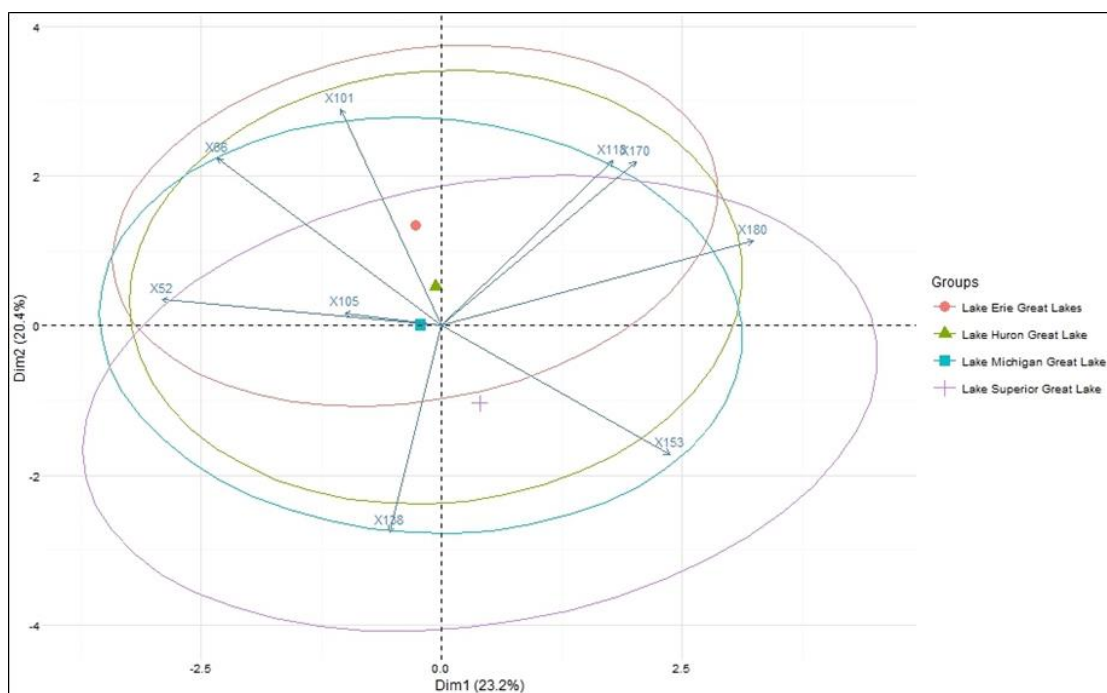


Figure 2.2 Principle Component Analysis (PCA) and 95% confidence ellipses for center of gravity polychlorinated biphenyl (PCB) congeners detected in at least 50% of bald eagle (*Haliaeetus leucocephalus*) plasma samples collected in Michigan from 1999-2014 for each Michigan (USA) Great Lakes spatial region: Lake Erie, Lake Huron, Lake Michigan, and Lake Superior.

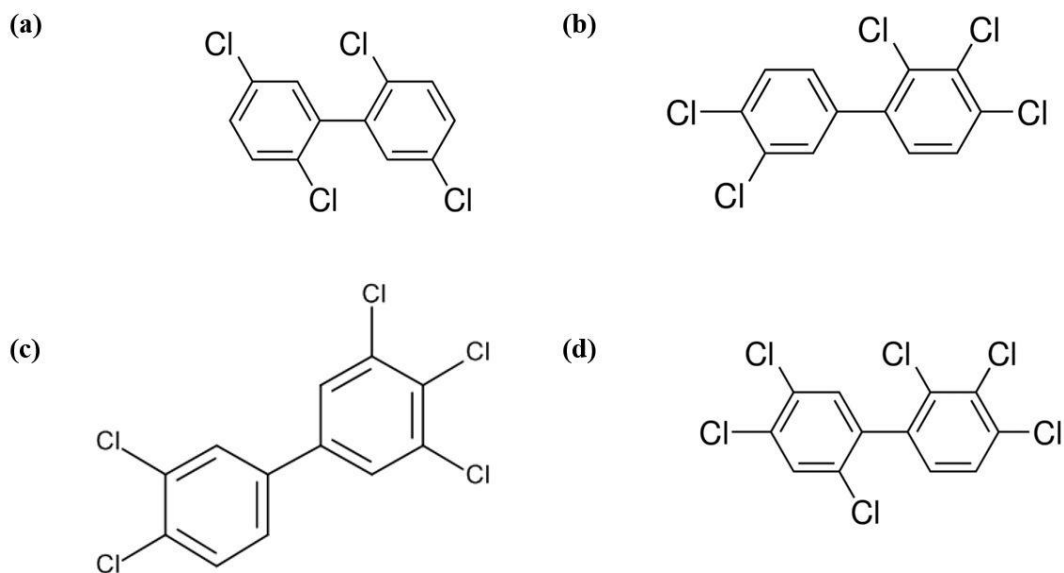


Figure 2.3 Structures of polychlorinated biphenyl (PCB) congeners (a) 52, (b) 105, (c) 126, and (d) 138.

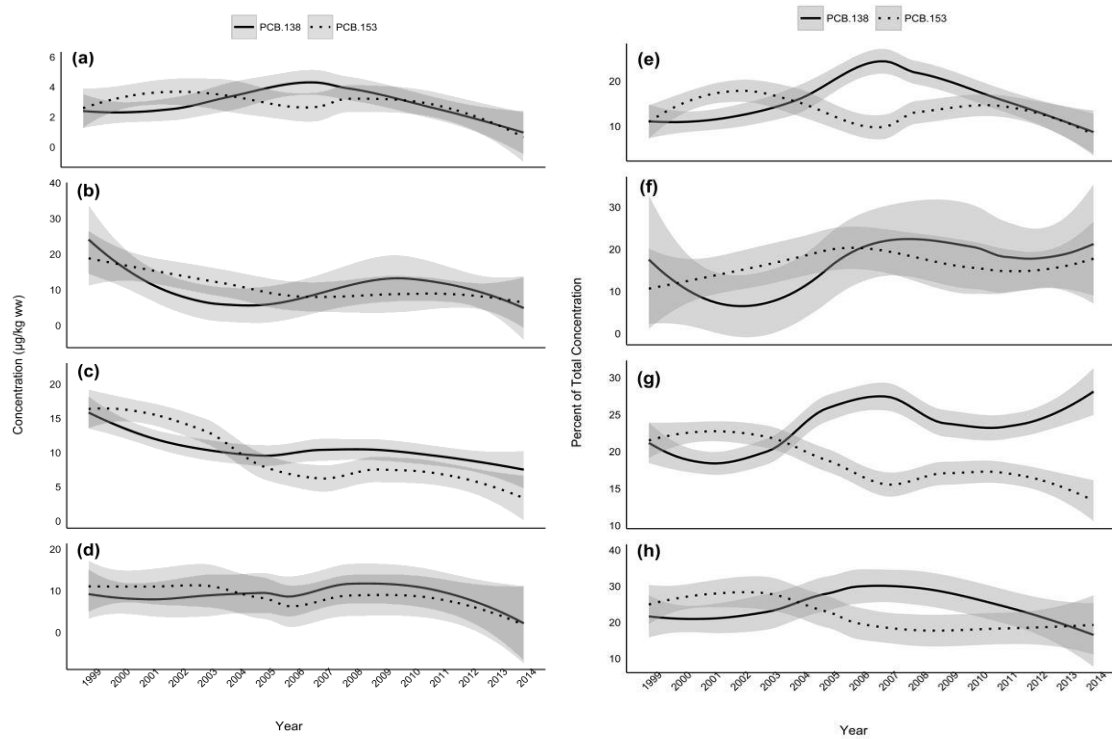


Figure 2.4 Trend lines of concentrations of polychlorinated biphenyl (PCB) congeners 138 and 153 for Michigan (USA) **(a)** Inland; **(b)** Lake Erie; **(c)** Lake Huron and Lake Michigan; and **(d)** Lake Superior Great Lakes spatial regions and of percent of total polychlorinated biphenyl (PCB) concentrations for congeners 138 and 153 for **(e)** Inland; **(f)** Lake Erie; **(g)** Lake Huron and Lake Michigan; and **(h)** Lake Superior Great Lakes spatial regions in bald eagles (*Haliaeetus leucocephalus*) from 1999–2014. Shaded areas are the 95% confidence region.

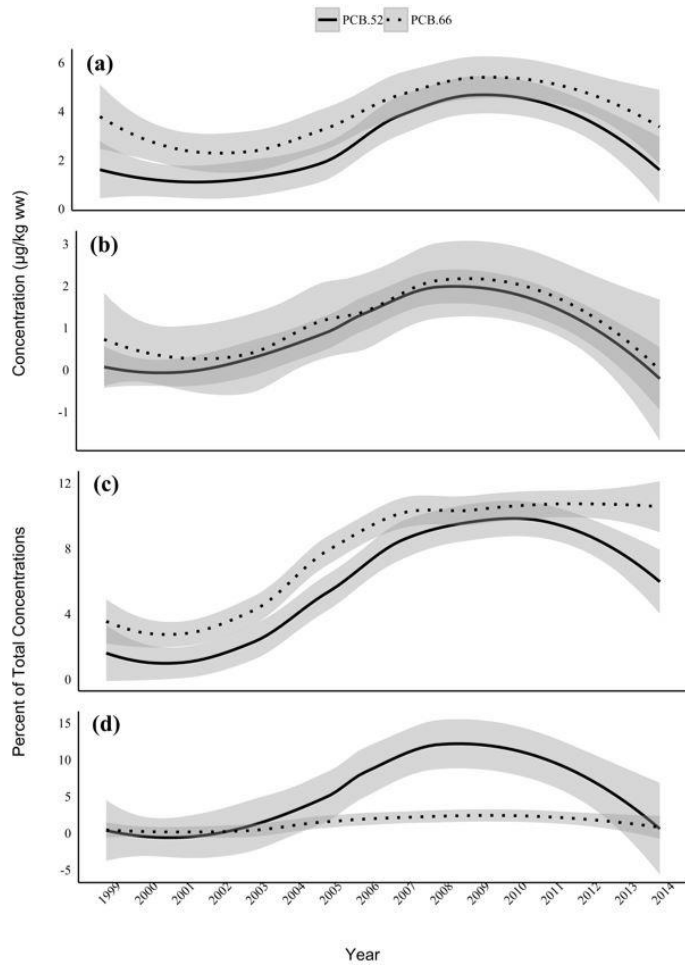


Figure 2.5 Trend lines of concentrations of polychlorinated biphenyl (PCB) congeners PCB 52 and PCB 66 for Michigan (USA) (a) Lake Huron and Lake Michigan and (b) Lake Superior Great Lake spatial regions and percent of total polychlorinated biphenyl (PCB) concentrations for congeners 52 and 66 for (c) Lake Huron and Lake Michigan and (d) Lake Superior Great Lakes spatial regions in bald eagles (*Haliaeetus leucocephalus*) from 1999–2014. Shaded areas are the 95% confidence region.

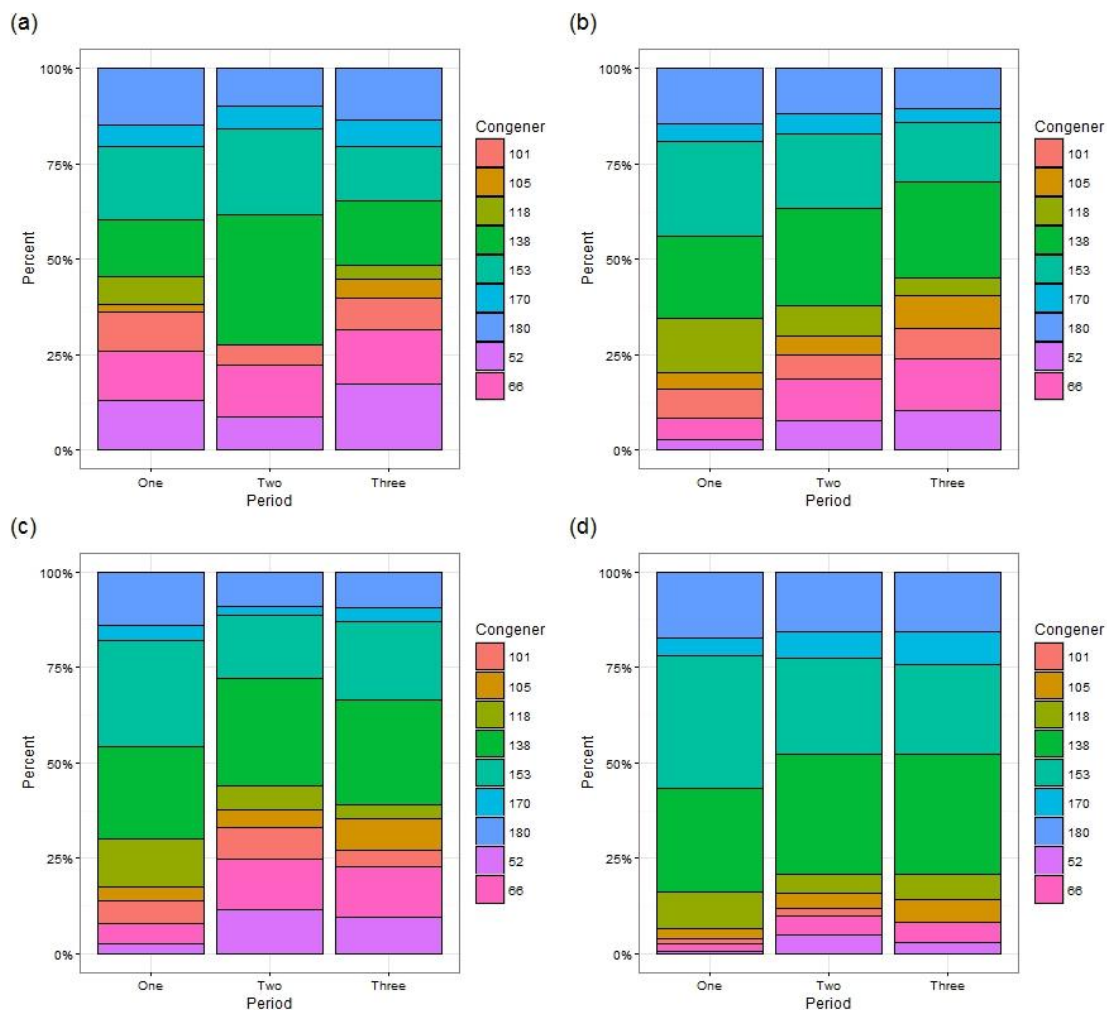


Figure 2.6 Percent of polychlorinated biphenyl (PCB) congener contribution in Michigan (USA) (a) Lake Erie, (b) Lake Huron, (c) Lake Michigan, and (d) Lake Superior Great Lakes spatial regions in bald eagles (*Haliaeetus leucocephalus*) for Periods: One (1999–2005), Two (2006–2009), and Three (2010–2014).

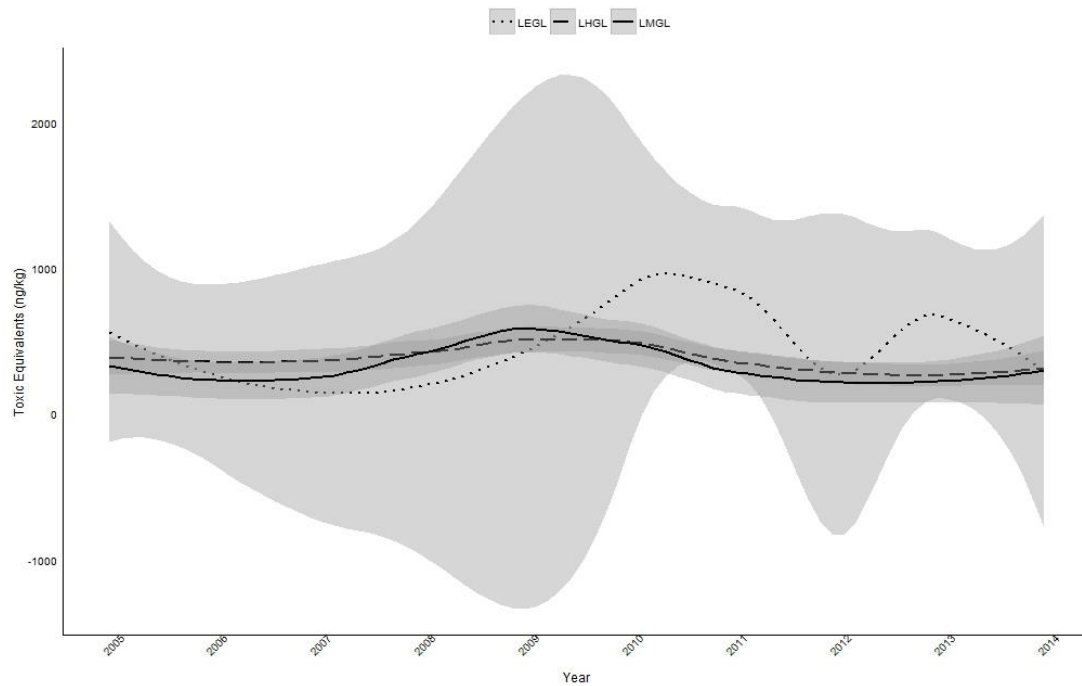


Figure 2.7 Trend lines of toxic equivalents for Michigan (USA) Lake Erie (LEGL), Lake Huron (LHGL), and Lake Michigan (LMGL) Great Lake spatial regions in bald eagles (*Haliaeetus leucocephalus*) from 2005–2014. Shaded areas are the 95% confidence region.

Chapter 3: Historic and Alternative Flame Retardants in Michigan Bald Eagles (*Haliaeetus leucocephalus*)

Introduction

Flame retardants are a wide range of compounds manufactured to delay ignition and reduce the flammability of commercial products such as plastics, foams, and furniture. Manufacturers began to add flame retardants to many household products following increased regulation or higher standards for protection in the early 1970's, such as the California flammability standard (Venier et al. 2015). Although flame retardants have successfully lowered the incidence of harm and economic costs of fires, concern has grown regarding their ubiquitous presence in the environment and subsequent toxicological effects (Birnbaum and Staskal 2004). The flame retardant industry voluntarily halted the production and sale of the most frequently-detected group of flame retardants, brominated diphenyl ethers (BDEs), following the overwhelming scientific evidence of their occurrence and concern as environmental pollutants (Jones and De Voogt 1999; Route et al. 2014). A cycle now seems to be occurring between the flame retardant industry and environmental scientists, as groups of flame retardants are detected in the environment, voluntarily taken off the market, and replaced with a newly developed group of flame retardant compounds that are aimed to be less persistent and bioavailable (Venier et al. 2015; Venier et al. 2010).

BDEs have been found in the sediment (Zhu and Hites 2005), air (Melymuk et al. 2014; Venier et al. 2012), biota (Chen and Hale 2010; Hahm et al. 2009; Su et al.

2015), and people (Hites 2004; Johnson-Restrepo and Kannan 2009). These compounds are of concern due to their environmental persistence (Covaci et al. 2011), and ubiquitous detection, even in remote areas (Dykstra et al. 2010; Route et al. 2014; Venier and Hites 2008). Measurable amounts of BDEs have been detected in avian eggs collected along the Great Lakes (Batterman et al. 2007; Chen et al. 2012; Gauthier et al. 2007). Great Lakes BDE concentrations, particularly the penta- and octa-BDE congeners, increased in fish and fish-eating birds until the mid-1990's, then began to plateau by the 2000's (Gauthier et al. 2008a). The global phase-out and listing of BDEs as persistent organic pollutants (POPs) by the Stockholm Convention in 2009 (UNEP 2009) has created a need for replacement flame retardant compounds. Many of these compounds, such as organophosphate esters (OPs) and Dechloranes, have been in use since the 1960's or 1970's, but are now produced to a greater extent to fill flame retardant requirements (Gandhi et al. 2015; Meijers and Van Der Leer 1976; Sheldon and Hites 1978; Shen et al. 2011a; Tachikawa et al. 1975). Following the phase out of the penta-brominated diphenyl ether (Penta-BDE) mixture, production volumes for three OP flame retardants, tris(1,3-dichloro-2-propyl) phosphate (TDCPP), triphenyl phosphate (TPP), and tris(2-chloro-isopropyl) phosphate (TCPP), increased from 455–4,536 metric tonnes in 1990 to 4,536–22,680 metric tonnes in 2006 in the United States (EPA).

Dechlorane analogues are also produced at greater volumes to fulfill flame retardant requirements and replace the parent compound, mirex. Mirex was used as a pesticide for about one-quarter of its production, and the remainder as a flame retardant under the name Dechlorane (Council 1978). Mirex was manufactured and

processed from 1957 to 1976 by the Hooker Chemical Co. (currently OxyChem) in Niagara Falls, NY and the Armstrong Cork Company near Fulton, NY, until its ban in the United States in the 1970's (Comba et al. 1993; Velleux et al. 1995). The adverse environmental characteristics of mirex also resulted in global bans and phase outs under the Stockholm Convention on POPs in April 2001 (Murphy et al. 2012; Shen et al. 2010). Similar analogues, such as Dechlorane Plus, Chlordene Plus, and Dechloranes 602, 603, and 604, were patented as replacements by OxyChem from the late 1960's to the mid 1980's.

Bald eagles (*Haliaeetus leucocephalus*) serve as ideal indicators, providing insight for tertiary-level exposure of contaminants transported to Great Lakes aquatic ecosystems largely via atmospheric deposition (Bowerman et al. 2002; Venier et al. 2010). Sampling nestling plasma also limits the scale of contamination to local areas as they are fed by adults within a territory of 4–5 km² in size from the breeding area (Garrett et al. 1993; Watson 2002). Only a handful of studies however, have reported on flame retardant concentrations in bald eagles. Dykstra et al. (2005) reported a geometric mean BDE concentration of 7.9 ng/mL (95% CI = 6.0–10.4) in five nestlings on Lake Superior in 2000 and 2001. Venier et al. (2010) reported ΣBDE levels of 15 bald eagle nestlings sampled in Michigan in 2005 ranging from 0.35–29.3 ng/mL (mean 5.7 ng/mL and nondetections omitted). This study also detected a few emerging flame retardants such as pentabromoethylbenzene (PBEB), hexabromocyclododecanes (HBCDs), and Dechlorane Plus (DP) (Venier et al. 2010). A third study conducted by Route et al. (2014) examined BDE patterns and trends in 284 bald eagle nestlings at six study areas in the upper Midwestern U.S. between

1995 and 2011. Geometric mean concentrations of Σ BDEs ranged from 1.78 ng/ mL and 12.0 ng/ mL. Patterns of greater BDE concentrations varied widely, but were mainly attributed to effluent from wastewater treatment plants (WWTP), and possibly a landfill. The authors argued that availability of BDEs to eagles was influenced by the physical and biological characteristics of the aquatic system (Great Lakes versus flowing rivers) in proximity to the nest (Route et al. 2014).

To further investigate the presence of not only BDEs, but also the ubiquity of emerging flame retardants in the Great Lakes, we used archived plasma samples of 6 to 9 week old bald eagle nestlings in four spatial regions of Michigan between 2000 and 2012. These samples were collected through the Wildlife Biosentinel Monitoring Project that was implemented by the Michigan Department of Environmental Quality. Our objectives in this study were to evaluate the occurrence and concentrations of historic and emerging flame retardants of concern, as well as to determine differences in flame retardant levels among spatial regions of varying contamination and water-level management backgrounds.

Methods

Field Methods

Samples were chosen from the Michigan Bald Eagle Biosentinel Program archive, which had nestling plasma samples and addled eggs collected during a single visit from 24 bald eagle nests within the state of Michigan from 2000 to 2012. To date, only plasma samples have been analyzed and are reported for this study. When originally collected, nestlings were temporarily removed from the nest, and up to

12mL of whole blood were taken from the brachial vein along with multiple morphometric measurements for age and sex calculations (Bortolotti 1984a; Bortolotti 1984b; Bowerman et al. 1995). When nests contained multiple nestlings in a single year, we randomly selected one plasma sample for the extraction process. Whole blood was stored on ice for no more than 48 hours before it was centrifuged, and plasma was pipetted into separate glass tubes for storage at approximately 20°C. All field procedures were conducted in accordance with the Animal Use Protocols of Clemson University (30067 & AUP2009-005) and the University of Maryland (744587-2), as well as the United States Geological Survey Bird Banding permit, and scientific collecting permits of the United States Fish and Wildlife Service and the Michigan Department of Natural Resources. All procedures performed in this study were in accordance with the ethical standards of the University of Maryland's Institutional Animal Care and Use Committee.

Samples chosen from the Michigan Bald Eagle Biosentinel Program archive were located from four spatial regions determined by known contamination levels and aquatic management techniques. Six samples were chosen for the Lake Superior Great Lake (LS) spatial regions. These samples were collected from nestlings in breeding areas along the Lake Superior shoreline, areas known to be relatively less contaminated areas than other Great Lakes samples for organochlorine pesticides and polychlorinated biphenyls (PCBs). Six samples were chosen for the Impoundment (IMP) spatial region. These samples were collected from nestlings in breeding areas located along waterbodies in the southwestern Upper Peninsula of Michigan. Breeding areas are located on rivers, lakes, ponds or reservoirs in which water levels

are artificially managed. The potential for sediment in impounded waterbodies to harbor elevated levels of contaminants may be high depending on many factors including the usage of the watershed, location of the impoundment, dredging history, and composition of the sediments. Contaminants previously sequestered in impoundments may be discharged downstream during major storm and flooding events. In essence, the impoundment shifts from acting as a contaminant sink to a contaminant source during these events. Therefore, comparing concentrations of flame retardants in samples collected on these impoundments to free-flowing waterbodies is of interest.

Six samples were chosen for the Menominee River (MMR) spatial region. These samples were collected from nestlings in breeding areas located along the Menominee River, which divides the Upper Peninsula of Michigan from northeastern Wisconsin. The main stem of the river flows between the cities of Menominee, Michigan and Marinette, Wisconsin before emptying into Green Bay. The Menominee River is designated an International Joint Commission Area of Concern (AOC), under the Great Lakes Water Quality Agreement, due to multiple sources of pollution including the a manufactured gas plant “Coal Tar Site”, chemical and ship building companies, two paper mills, two municipal wastewater treatment plants, a foundry, runoff from stormwater, and storage piles of salt and coal.

Lastly, six samples were chosen for the Saginaw Bay (SGB) spatial region. These samples were collected from nestlings located along the shorelines of the Saginaw Bay, Lake Huron, or anadromous river that are open to Lake Huron fish passages (Figure 3.1). Breeding areas are located in the east central portion of

Michigan's Lower Peninsula, and are a southwestern extension of Lake Huron. The Saginaw Bay region is also considered an AOC due to contaminated sediments, fish consumption advisories, degraded fisheries, and loss of significant recreational values, primarily from nonpoint sources discharges, combined sewer overflows, sanitary sewer overflows, and industrial sources of PCBs. Other major contaminants in the AOC include dioxins, furans, chloride, metals, acids, and excessive nutrients, such as nitrogen and phosphorus.

Due to past findings of greater organochlorine contaminant levels in bald eagle nestlings located along the Great Lakes' shorelines (Bowerman et al. 2003; Bowerman et al. 2002), we also divided the samples between Inland (IN) breeding areas and Great Lakes (GL) breeding areas. Breeding areas located greater than 8.0 km from the shorelines of the Great Lakes, and not along tributaries where anadromous fish were accessible, are designated as IN. Breeding areas located less than 8.0 km from the shorelines of the Great Lakes, or along tributaries where anadromous fish were accessible, are designated as GL (Figure 3.1).

Materials

Florisil (Sigma-Aldrich, St. Louis, MO) was baked overnight at 300°C then deactivated with 2.5% (by weight) water once cooled to room temperature. It was then stored overnight in a desiccator. Anhydrous sodium sulfate (EMD Chemicals, Gibbstown, NJ) was baked overnight at 500°C and cooled to room temperature before use.

Individual non-BDEs standards [including tetrabromo-*p*-xylene (pTBX), PBBZ, TBB, TBPH, *syn*- and *anti*-DP], as well as a BFR-PAR solution mixture

[consisting of PBEB, HBB, 1,2-bis(2,4,6-tribromophenoxy)ethane(TBE), BDE-7, 10, 15, 17, 28, 30, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138–140, 153, 154, 156, 169, 180, 183, 184, 191, 196, 197, 201, and 203–209] were purchased from Wellington Laboratories (Guelph, ON). Individual OP standards including *tris*(3,5-dimethylphenyl)phosphate (TDMPP), *tris*(2-isopropylphenyl) phosphate (TIPPP), *tri*-*p*-tolyl-phosphate (TPTP), *tri*-*o*-tolyl-phosphate (TOTP), *tris*(2-ethylhexyl)phosphate (TEHP), 2-ethylhexyl-diphenyl-phosphate (EHDP), triphenyl phosphate (TPHP), *tris*(1,3-dichloro-2-propyl)phosphate (TDCIPP), *tris*(1-chloro-2-propyl)phosphate (TCIPP), *tris*(2-chloroethyl)phosphate (TCEP), *tri*-*n*-butylphosphate (TnBP) were also purchased from Wellington Laboratories. *Tris*(4-tert-butylphenyl) phosphate (TBPP) was purchased from Sigma-Aldrich (St. Louis, MO).

Surrogate recovery standards for BDEs were BDE-77 and BDE-166 from AccuStandard (New Haven, CT), and C₁₂-BDE-209 from Wellington Laboratories. Surrogate recovery standards for OPs were *tris*(2-chloroethyl) phosphate-*d*₁₂ from Sigma-Aldrich and C₁₈-triphenyl phosphate from Wellington Laboratories. Internal quantitation standards for BDEs were BDE-118 from AccuStandard, and BDE-181 and BB-209 from Wellington Laboratories. Internal quantitation standards for OPs were the deuterated PAH standards, anthracene-*d*₁₀, dibenz[*a*]anthracene-*d*₁₂, and perylene-*d*₁₂. These were purchased from Chem Service (WestChester, PA).

Analytical Procedures

The entire sample of plasma (ranging from 2–5.4 mL) was weighed, spiked with known amounts of surrogate recovery standards, denatured with 2 mL of 6M HCL and 5 mL of 2-propanol, and liquid-liquid extracted with 10 mL of

hexane/methyl *t*-butyl ether (1:1). One or two procedural blanks, using 3.5 mL of water, were also included in every batch. The tubes were shaken for 20 minutes then centrifuged for 10 minutes at 3000 rpm. The upper organic layer was transferred to a pear shape flask. The extraction step was repeated twice, combining all organic layers. Upon completion of the extraction steps, the lipid content was determined by removing 1 mL of the organic extract into a pre-weighed aluminum disc and re-weighed the following day, allowing the solvent to evaporate. The organic extract was then rotary evaporated to 1 mL with on solvent change of 25 mL hexane. One-cm diameter columns were prepared with 6g of 2.5% (by weight) water deactivated Florisil, followed by 4g anhydrous sodium sulfate. Columns were rinsed with 40 mL of hexane. Samples were loaded into columns and eluted with three fractions with a separate pear shape flask for each. The first fraction was 35 mL of hexane, the second was 35 mL of hexane:dichloromethane (1:1 vol), and the third fraction was 40 mL of dichloromethane:acetone (1:1 vol). Based on the results of Liu et al. 2015, most of the BDEs and some NBFRs elute in the first fraction. TBE, TBB, and TBPH however, elute in the second fraction. All OPs elute in the third fraction. All fractions in pear shape flasks were rotary evaporated down to 1 mL. The first fraction did not have any solvent changes. The second fraction had one solvent change of 25 mL hexane and the third fraction had two solvent changes of 25 mL hexane each. Fractions were transferred to 4 mL vials, blown down to 1 mL with N₂, and spiked with known amounts of internal standards. Internal standards for the first and second fractions included BDE-118, BDE-181, and BB-209. Internal standards for the third fraction

included anthracene-*d*₁₀, dibenz[*a*]anthracene-*d*₁₂, and perylene-*d*₁₂. The first and second fractions were then blown down to 100 µL with N₂ (Liu et al. 2014).

Here is a brief summary of instrumental analysis. Further detailed explanations can be found in Peverly et al. (2014) and Liu et al. (2014). Fractions one and two were analyzed for BDEs and NBFRs with an Agilent 7890 series gas chromatograph (GC) in pulsed splitless mode, coupled to an Agilent 5975 GC mass spectrometer (MS) in the electron capture negative ionization mode. High purity helium was used as the carrier gas, and methane was used as the reagent gas. A Rtx-1614 (15 m × 250 µm i.d., 0.1 µm film thickness) fused silica capillary GC column was used to achieve chromatographic resolution (Restek Corporation, Bellefonte, CA). One µL of the sample was injected at a time. Inlet temperatures were maintained at 240°C. The GC oven temperature was initially held at 100°C for 2 minutes, ramped to 250°C at 25°C/min, to 270°C at 3°C/min, to 320°C at 25°C/min, and held there for 9 min.

Fraction three was analyzed for OPs with an Agilent 6890 series GC in pulsed splitless mode at 280°C, coupled to an Agilent 5973 MS operating in the electron impact mode. A 30 m × 250 µm i.d. (0.25 µm film thickness) DB-5MS Ultra Inert capillary column (Agilent Technologies, Santa Clara, CA) was used to achieve chromatographic resolution. One µL of the sample was injected at a time. The temperature of the ion source and the GC was maintained at 230°C, and the MS transfer line at 300°C. High purity helium was used as the carrier gas. The GC oven temperature was held at 70°C for 3 min, increased to 170°C at 10°C/min, held for 5

min, to 230°C at 10°C/min, held for 5 min, to 250°C at 5°C/min, then to 300°C at 10°C/min, and held there for 4 min.

Statistical Methods

All analyses were conducted in R (R Development Core Team 2015). Comparisons of total brominated diphenyl ethers (BDEs), non-polybrominated diphenyl ethers flame retardants (NBFRs), dechlorane plus (DPs), and organophosphate esters (OPs) were tested for normality and homogeneity of variance, and log transformed as needed. Comparisons were made among spatial regions, as well as between IN and GL breeding areas, for total BDE, Dechlorane, and OP concentrations using analysis of variance (ANOVA), followed by a Tukey HSD multiple mean comparison test ($\alpha = 0.05$).

Statistical comparisons in patterns of flame retardant congener or compound compositions among spatial regions, and between IN and GL, were made using analysis of similarity (ANOSIM), a multivariate analog of analysis of variance, for BDEs, Dechloranes, and OPs. ANOSIM is built on a nonparametric permutation procedure and applied to the rank similarity matrix underlying the ordination of samples (Clarke 1999). The test statistic R ranges from -1 to +1, with zero meaning the distribution of patterns is as similar among groups as within groups and +1 meaning there are very clear differences in patterns among the groups being tested, in this case spatial regions or IN and GL breeding areas. An R value of ≥ 0.4 indicated some support for pattern differences and an R value of < 0.3 indicated little difference (Custer et al. 2010). Only congeners in which at least 50% of the samples were above the limits of quantification by the GC were used for ANOSIM analyses and reported

in Table 3.1. Concentrations below the limits of quantification by the GC were replaced with half of the detection limit found for each individual congener. Due to the large number of decimal values resulting in negative values following a log transformation, contaminant data were $\log(x+1)$ transformed prior to ANOSIM analyses. Outliers were removed from the dataset using a Walsh's test with an α level of 0.05.

The Peto-Prentice version of the generalized Wilcoxon (Gehan) test, accounting for censored values, was used to determine whether the distribution of total concentrations of NBFRs differed significantly among spatial regions, and between IN and GL breeding areas. This was also used to determine differences among individual Dechlorane compounds between IN and GL breeding areas (Lee 2013). If the p -value from the two-group score tests was less than the Bonferroni-adjusted individual comparison level calculated as:

$$\text{individual error rate} = \frac{\alpha}{g}$$

where α is the overall error rate (0.05) and g is the number of comparisons to be made (Helsel 2011).

Results

BDEs

The following congeners were measured in bald eagle plasma samples: BDE-7, 10, 15, 17, 28, 30, 47, 49, 66, 71, 85, 99, 100, 119, 126, 138, 139, 140, 153, 154+BB153, 156+169, 180, 183, 184, 191, 196, 197, 201, 203, 204, 205, 206, 207,

208, 209. Of these, BDE-205 was the only congener that was not detected in any samples. The congeners in which less than 50% of samples were below the limit of detection, and therefore used for ANOSIM statistical analyses, were BDE-7, 15, 17, 28, 47, 49, 66, 85, 99, 100, 119, 126, 140, 153, 154+BB153, 184, 201, 208, 209. Congeners that were quantifiable in 100% of the samples were BDE-17, 28, 47, 49, 99, 100, 126, 153, 154+BB153, and 201 (Table 3.1). Concentrations of Σ BDEs ranged from 0.48–22.49 ng/g ww (wet weight), with a geometric mean of 4.64 ng/g ww. Congeners BDE-47, 99, and 100 were the greatest contributors, representing an average of 45%, 21%, and 10%, respectively (Figure 3.3a). The sum of these three congeners represents 76% of Σ BDE levels. The highly brominated congener, BDE-209, was detected in 67% of samples with concentrations ranging from <MDL–2.59 ng/g ww.

There was an overall difference in geometric mean concentration of Σ BDEs among spatial regions (ANOVA, $F = 3.22$, $p = 0.0445$). Geometric mean concentrations were greatest in the SGB spatial region (6.72 ng/g ww), and similar to MMR and LS spatial regions (6.69 and 6.20 ng/g ww, respectively). Mean concentrations were least for the IMP spatial region (1.67 ng/g). No statistically significant differences were found among spatial regions using Tukey's HSD mean separation test (Table 3.2). Biologically significant differences however, may be inferred between IMP and MMP spatial regions ($p = 0.77$) as well as IMP and SGB spatial regions ($p = 0.076$).

No differences in congener distributions were found among spatial regions as well (ANOSIM $R = 0.1765$, $p = 0.029$). There was also no overall difference in

geometric mean concentration of Σ BDEs between IN and GL breeding areas (ANOVA, $F = 3.87$, $p = 0.062$; Table 3.3). No differences in congener distributions were found between IN and GL breeding areas as well (ANOSIM $R = 0.1917$, $p = 0.023$). Because of this, concentrations of individual BDE congeners were not reported separately for spatial regions or IN and GL breeding areas (Table 3.1). Only Σ NBFR concentrations are reported separately for spatial regions, as well as IN and GL breeding areas.

NBFRs

The following NBFR compounds were measured in bald eagle plasma samples: pentabromotoluene (pTBX), pentabromobenzene (PBBz), pentabromoethylbenzene (PBEB), hexabromobenzene (HBB), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB or EHTBB), hexabromocyclododecane (HBCD), 1,2-bis(2,4,6-tribromo-phenoxy)ethane (TBE), bis(2-ethylhexyl)-3,4,5,6-tetrabromophthalate (TBPH or BEHTBP), decabromodiphenyl ethane (DBDPE). Of these compounds, pTBX, PBEB, HBB, and TBE were not detected in any of the samples. Concentrations of Σ NBFRs ranged from $<\text{MDL}-1.249$ ng/g ww, with a geometric mean of 0.179 ng/g ww. DBDPE the greatest contributor (55%) to Σ NBFR concentrations, followed by TBPH (22%) which was only detected in two SGB samples (Table 3.1; Figures 3.2b and 3.3).

There was there was no overall difference in geometric mean concentrations of Σ NBFRs among spatial regions (Chi Square = 2.7, $p = 0.448$; Table 3.2). Because of this, concentrations of individual NBFR compounds were not reported separately for spatial regions (Table 3.1). DBDPE was the only compound in which less than

50% of the samples were below the limit of detection. Because of this, differences in congener contribution were not analyzed using ANOSIM. There was also no overall difference in geometric mean concentration of Σ NBFRs between IN and GL breeding areas (Chi Squared = 2.3, $p = 0.134$; Table 3.3). Because of this, concentrations of individual NBFR compounds were not reported separately for spatial regions or IN and GL breeding areas (Table 3.1). Only Σ NBFR concentrations are reported separately for spatial regions, as well as IN and GL breeding areas.

Dechloranes

The following Dechlorane compounds were measured in bald eagle plasma samples: *syn*-Dechlorane Plus (*syn*-DP), *anti*-Dechlorane Plus (*anti*-DP), *syn*- and *anti*-Dechlorane Plus combined (total DP), hexachloro(phenyl)-norbornene (HCPN), 1,3-Dechlorane Plus-monoadduct (1,3-DPMA), 1,5-Dechlorane Plus-monoadduct (1,5-DPMA), Chlordane (Kepone), Dechlorane (Mirex), Dechlorane 602 (Dec602), Chlordane Plus (CP), Dechlorane 604 component B (Dec604 CB), Dechlorane 603 (Dec603), 2,3,4,5-tetrabromophenyl-hexachloro-norbornene (Dec604), decachloropentacyclooctadecadiene (CL₁₀DP), Dechlorane 601 (Dec601), undecachloropentacyclooctadecadiene (CL₁₁DP), Monobromophenyl-hexachloro-norbornene (BrDec604-1a), monobromophenyl-hexachloro-norbornene (BrDec604-1b), monobromophenyl-hexachloro-norbornene (BrDec604-1c), 3,5-dibromophenyl-hexachloro-norbornene (Dec604-2), tetrachlorophenyl-hexachloro-norbornene (Dec604-Cl₄), 2,4,6-tribromophenyl-hexachloro-norbornene (Br₃Dec604), 4,6-dibromo-2,3-dichlorophenyl-hexachloro-norbornene (Br₂Cl₂Dec604). Of these compounds, Kepone and Dec601 were not detected in any samples. The congeners in

which less than 50% of samples were below the limit of detection, and therefore used for ANOSIM statistical analyses, were *syn*-DP, *anti*-DP, total DP, HCPN, 1,3-DPMA, 1,5-DPMA, Mirex, Dec602, CP, Dec604 CB, Dec603, BrDec604-1a, BrDec604-1b, BrDec604-1c, Dec604-Cl₄, Br₂Cl₂Dec604. Dechlorane compounds that were quantified in 100% of the samples were total DP, HCPN, 1,3-DPMA, Mirex, Dec602, and Dec 603. Concentrations of Σ Dechloranes ranged from 0.101–6.917 ng/g ww, with a geometric mean of 1.294 ng/g ww. BrDec 604-1a and BrDec 604-1b were the two greatest contributors (24 and 23%, respectively) to Σ Dechlorane concentrations, followed by Mirex and Br₂Cl₂Dec604 (11% each; Figures 3.2c and 3.4).

There was there was no overall difference in geometric mean concentrations of Σ Dechloranes among spatial regions (ANOVA $F = 2.00$, $p = 0.147$; Table 3.2). No differences in compound distributions were observed among spatial regions (ANOSIM $R = 0.2423$, $p = 0.003$). Because of this, concentrations of individual Dechlorane compounds were not reported separately for spatial regions. There was there was an overall difference in geometric mean concentrations of Σ Dechloranes between IN and GL breeding areas (ANOVA $F = 13.47$, $p = 0.0013$; Table 3.3), with greater levels in GL breeding areas (2.63 ng/g ww) than IN breeding areas (0.711 ng/g ww). Differences were also found in compound distributions between GL and IN breeding areas (ANOSIM $R = 0.4386$, $p = 0.001$; Figure 3.4). Because of this, concentrations of individual Dechlorane compounds were reported separately for IN and GL breeding areas (Table 3.4). Significant differences in geometric mean

concentrations between IN and GL breeding areas were found for HCPN, 1,3-DPMA, Mirex, CP, Dec602, Dec603, BrDec604-1a, and Dec604-Cl₄ (Table 3.4).

OPs

The following OPs were measured in bald eagle plasma samples: tri-isopropyl phosphate (TIPRP), tri-n-propyl phosphate (TPRP), tri-n-butyl phosphate (TnBP), tris(2-chloroethyl) phosphate (TCEP), tris (2-chloroisopropyl) phosphate (TCPP or TCIPP), triphenyl phosphate (TPP or TPhP), tris(1,3- dichloro-2-propyl) phosphate (TDCPP or TDCIPP), tris(butoxyethyl)phosphate (TBEP or TBOEP), 2-Ethylhexyl diphenyl phosphate (EHDP), tris(2-ethylhexyl) phosphate (TEHP), tri-o-tolyl phosphate (TOTP), tri-p-tolyl phosphate (TPTP), tris-(2-isopropylphenyl) (TIPPP), tris(3,5-dimethylphenyl) phosphate (TDMPP), and tris(4-tert-butylphenyl) phosphate (TBPP). Of these compounds, TCEP, TOTP, and TDMPP were not detected in any samples. The congeners in which less than 50% of samples were below the limit of detection, and therefore used for ANOSIM statistical analyses, were TIPRP, TPRP, TnBP, TCPP, TPP, TPP, and TIPPP. Compounds that were quantifiable in 100% of the samples were TPRP, TnBP, and TPEP. Concentrations of Σ OPs ranged from <MDL–53.11 ng/g ww, with a geometric mean of 18.14 ng/g ww. TPP and TIPPP were the two greatest contributors (26 and 20%, respectively) to Σ OP concentrations (Figures 3.2d and 3.3c).

There was there was no overall difference in geometric mean concentration of Σ OPs among spatial regions (ANOVA $F = 0.13$, $p = 0.94$; Table 3.2). No differences in compound distributions were observed among spatial regions (ANOSIM $R = 0.004784$, $p = 0.402$). Because of this, concentrations of individual OP compounds

were not reported separately for spatial regions. There was also no overall differences in geometric mean concentration of ΣOPs between IN and GL breeding areas (ANOVA, $F = 0.08$, $p = 0.7774$; Table 3.3). No differences in compound distributions were found between IN and GL breeding areas as well (ANOSIM $R = -0.09143$, $p = 0.984$). Because of this, concentrations of individual OP compounds were not reported separately for spatial regions or IN and GL breeding areas (Table 3.1). Only ΣOP concentrations are reported separately for spatial regions, as well as IN and GL breeding areas.

Discussion

BDEs

ΣBDE concentrations were similar to past studies in bald eagle plasma. Venier et al. (2010) reported concentrations in plasma samples collected along the shorelines and drainages of Lakes Superior, Michigan, and Huron ranging from 0.35–29.3 ng/g ww, with an average of 5.7 ± 1.9 ng/g ww. Dykstra et al. (2005) reported a greater average concentration of 7.9 ng/g ww, but a lesser range of 6.0–10.4 ng/g ww in bald eagle plasma collected along the shoreline of Lake Superior from 1989 to 2001. Our BDE concentrations are also similar to those measured in nestling bald eagle plasma from British Columbia. McKinney et al. (2006) also reported lesser concentrations ranging from 0.40–8.5 ng/g ww in samples collected in 2001 and 2003, but a mean value of 30.9 ng/g in three nestlings on Santa Catalina Island, CA. Our total geometric mean BDE concentrations for the IMP spatial region are comparable to those reported by Route et al. (2014) in the Upper St. Croix National Scenic River

(1.78 ng/g, range = 1.53–2.07 ng/g). These levels may reflect the inaccessibility of these waterways to Great Lakes fish runs. Route et al. (2014) also observed a 4-fold increase in plasma BDE levels downstream of densely populated, industrialized areas in the St. Croix River and into the Mississippi River, as well as on islands within, or shorelines along Lake Superior. Similar to this, our results indicate greater BDE levels along the shores of the Great Lakes in the LS spatial region, and within densely populated areas, such as the city of Saginaw, within the SGB spatial regions. Previous studies in the Great Lakes region attributed the pattern of increasing downstream BDE levels to effluent from wastewater treatment plants (WWTPs)(Route et al. 2014). This association of greater BDE levels proximate to WWTPs has also been documented in other studies (Liu et al. 2014; Melymuk et al. 2014). Four WWTPs are proximate to the SGB nests that were sampled; the Midland WWTP, the Au Gres WWTP, the Bay City WWTP, and the Frankenmuth WWTP (Figure 3.1), possibly explaining the greater Σ BDE levels in this region. Greater Σ BDE levels in nestlings within the MMR may also be partially explained by the Menominee River, Marinette, and Iron Mountain-Kingsford WWTPs, but also by Tyco Fire Suppression Systems, which specializes in special fire-hazard protection products, located on the south side of the Menominee Harbor (Figure 3.1). The greater concentrations of Σ BDEs in nestling plasma sampled within the LS spatial region could be due to the large drainage area, coupled with the cold water temperatures and slow decomposition rates, as suggested by Route et al. (2014).

Individual congener contribution to Σ BDEs were also similar to past studies in bald eagle plasma. Venier et al. (2010) found that one tetra- and two penta-isomers,

BDE-47, 99, and 100, contributed 32%, 20%, and 16% (an average of 67% combined) of Σ BDE concentrations, respectively. Route et al. (2014) and McKinney et al. (2006) both found that BDE-47 contributed roughly half of the Σ BDE concentrations, followed by BDE-99, and 100. BDE-209 was detected in greater than 50% of samples (15 of 24), ranging from <MDL–2.59 ng/g ww and a mean ranging from 0.0257–0.2428 ng/g. Of these 15 samples, six were collected between 2005 and 2012. This prevalence of detection is greater than that reported by Venier et al. (2010), in which BDE-209 was detected in 4 of 15 samples collected in 2005. This can be partially attributed to the industrial replacement of penta- and octa-BDE formulations with deca-BDEs (consisting predominately of BDE-209) and the propensity of BDE-209 to partition to sediment in abiotic environments such as lakes, which then act as persistent reservoirs (Ross et al. 2009). For instance, BDE-209 was found to be the dominant congener in sediment cores collected in 2003 and 2004 in Lakes Erie and Michigan, comprising ~95% of the 10 and 23 metric tons of Σ BDE loads, respectively (Zhu and Hites 2005). Although deca-BDE was voluntarily phased out in the United States by 2014, the presence of BDE-209 in nestling bald eagles suggests this congener may be of concern as *in vivo* debromination into hepta-, octa-, and nona-BDEs has been shown to occur either along the food web, or the in the birds themselves (Gauthier et al. 2008a; Holden et al. 2009; Pirard and De Pauw 2007; Van den Steen et al. 2007). Although not well studied, these lower-brominated congeners then have potential to cause adverse reproductive, developmental, and behavioral effects (Fernie et al. 2005a; Fernie et al. 2009; Fernie et al. 2008; Fernie et al. 2005b; Henny et al. 2009; Johansson et al. 2009; McKernan et al. 2009).

NBFRs

DBDPE was found to be the major contributor to the NBFR compounds, ranging from <MDL–0.564 ng/g ww and a geometric mean of 0.22 ng/g ww. DBDPE was introduced into the market in the early 1990's as an alternative to BDE-209 (Kierkegaard et al. 2004). It has been detected in tree bark and the atmosphere of the Great Lakes environment, and shown to be strongly and positively correlated with BDE-209 concentrations. (Liu et al. 2016; Ma et al. 2013; Salamova and Hites 2010; Salamova and Hites 2011; Venier and Hites 2008). Concentrations of DBDPE were also measured in egg pools of herring gulls (*Larus argentatus*) from seven colonies in the five Great Lakes (collected from 1982 to 2006). DBDPE concentrations were greatest in three of the seven colonies (1.3–288 ng/g ww) in 2005 and 2006, surpassing BDE-209 levels (Gauthier et al. 2008b).

TBPH was the second greatest contributor of Σ NBFR compounds, detected at 0.164 ng/g ww and 1.075 ng/g ww in two SGB samples. TBB was the second most frequently detected NBFR (10 of 24 samples), ranging from <MDL–0.173 ng/g ww. TBPH and TBB were used in a 4:1 ratio (by mass) mixture in the additive flame retardant product Firemaster 550. Firemaster 550 has been produced since 2003 by Chemtura Chemical Corporation as a replacement for penta-BDEs in polyurethane foam applications (Covaci et al. 2011). Strong, positive correlations were also found in atmospheric concentrations of TBPH plus TBB (representing Firemaster 550) and BDE-47, 85, 99, 100, 153, plus 154 (representing the withdrawn penta-BDE commercial mixture)(Ma et al. 2013). Atmospheric concentrations of TBPH and TBB in Cleveland are increasing significantly, with a doubling time of about 2 years. This

area more clearly shows the succession of BDEs by these alternative products (Venier et al. 2015). Minimal studies have detected TBPH and TBB in wildlife. Finless porpoise (*Neophocaena phocaenoides*) samples in coastal waters of Hong Kong, China contained TBPH and TBB at concentrations ranging from <0.04–3,859 ng/g lw, and <0.04–70 ng/g lw. Concentrations were also measured in dolphins, ranging from <0.04–5 ng/g lw for TBPH, but below the detection limit (<0.04 ng/g lw) for TBB (Lam et al. 2009). TBB and TBPH and TBB were also detected in Great Lakes fish at concentrations ranging from 0.04–0.08 ng/g, and 0.01 ng/g to 0.04 ng/g (unit basis not provided), respectively (Zhou et al. 2010).

HBCDs, reported as the sum of the three isomers, were detected in three samples within the LS spatial region, ranging from <MDL–0.175 ng/g ww. This detection frequency is lesser than those reported by Venier et al. (2010) in which it was detected in seven of 15 samples, at an average concentration of 0.13 ± 0.07 ng/g. HBCDs are used as an additive in extruded and expanded polystyrene foams, which are used as thermal insulation in buildings and upholstery textiles. HBCDs were largely used in Europe, and are now ubiquitous contaminants in the environment (Covaci et al. 2006). Mean concentrations of HBCD measured in the plasma of arctic-breeding glaucous gulls (*Larus hyperboreus*) were 2.7 ± 0.7 ng/g ww, with a direct relationship between females containing relatively high plasma proportions of total-(α)-HBCD and smaller eggs laid (Verboven et al. 2009). HBCDs, particularly the α -diastereomer exhibit a tendency to bioaccumulate and biomagnify (Haukås et al. 2010; Lundstedt-Enkel et al. 2006; Sun et al. 2012; Tomy et al. 2004). For these reasons, the Stockholm Convention has voted for a global ban of HBCD, listing it as

a persistent organic pollutant (POP) in 2013 (Committee 2013). PBBz was the only bromobenzene flame retardant detected in our study, ranging from 5.09–29.13 ng/g ww. No information is available for past or current uses of PBBz (Venier et al. 2012). PBBz has been detected in the Great Lakes atmosphere, as well as in tree bark in Ontario, Canada and at Košetice in the Czech Republic (Salamova and Hites 2012; Venier et al. 2012).

Dechloranes

The greater Dechlorane concentrations in GL breeding areas may be a result of multiple factors including local production facilities on waterways connected to the Great Lakes, current use of analogues, and physiochemical characteristics affecting environmental transfer and fate. Occidental Chemical Corporation (OxyChem) may be a proximate source of Dechlorane compounds as it originally produced Mirex, a persistent, bioaccumulative, and toxic organochlorine compound, followed by multiple derivatives or analogues. The greater occurrence of these subsequent compounds, particularly HCPN, 1,3-DPMA, CP, Dec602, Dec603, Dec604-1a, and Dec604-Cl₄, in the Great Lakes aquatic ecosystem may indicate their environmental behaviors are similar to Mirex.

Mirex was detected in 100% of samples, ranging from 0.008–1.892 ng/g ww and a geometric mean of 0.102 ng/g ww. A study determining mirex concentrations in Great Lakes fish reported the majority of measurements (except for Lake Ontario) were below detection, and concentrations in Lake Ontario decreased by approximately 90% between 1975 and 2010 (Gandhi et al. 2015). Mirex concentrations measured in bald eagle nestlings in 1999 ranged from 0.0001–0.0066

mg/kg ww (0.1–6.6 ng/g ww), with a means of 0.0006 and 0.0012 mg/kg ww (0.6 and 1.2 ng/g ww) for Lakes Erie and Superior, respectively (Donaldson et al. 1999).

Mirex concentrations measured in the plasma of arctic-breeding glaucous gulls collected in 2002 and 2004 ranged from 5.44–61.2 ng/g lw (0.044–0.49 ng/g ww), with an arithmetic mean of 22.2 ± 1.85 ng/g lw (0.18 ± 0.015 ng/g ww) in males, and 7.57–48.1 ng/g lw (0.06–0.35 ng/g ww), with an arithmetic mean of 21.9 ± 1.63 ng/g lw (0.18 ± 0.013 ng/g ww) in females (Verreault et al. 2005). Conversions from lipid weight to wet weight were based on a 0.8% plasma lipid content in nesting ring-billed gulls (*Larus delawarensis*) (Martinson et al. 2016).

Mirex was replaced by Dechlorane Plus (DP), Chlordene Plus (CP), Dechlorane (Dec) 602, 603, and 604, which are halogenated norbornene derivatives containing a basic bicyclo [2,2,1]-heptene structure with similar flame retardant properties (44 ; Shen et al. 2011a). Total DP (sum of the *-syn* and *-anti* isomers) was detected in 70% of samples (17 of 24), ranging from <MDL–1.11 ng/g ww, with a Kaplan-Meier estimate of the mean ranging from 0.022–0.11 ng/g ww. These concentrations are lower than those reported by Venier et al. (2010), who detected DP in 40% of samples bald eagle plasma samples (6 of 15) and an average concentration of 0.19 ± 0.10 ng/g ww. DP is an additive flame retardant in electrical hard plastic connectors and cable coatings (Sverko et al. 2011; Wang et al. 2016), and is industrially synthesized through a Diels–Alder reaction resulting in two diadduct isomers: *syn*- and *anti*-DP (Tomy et al. 2013). The *anti*-DP is about three times more abundant than *syn*-DP in the commercial product, as well as the environment (Venier et al. 2015). In agreement with this, we observed greater geometric means of *anti*-DP

(0.235 ng/g ww) compared to syn-DP (0.0096 ng/g ww). Although DP is considered a global contaminant, the main source for the Great Lakes region is in Niagara Falls, NY, where DP is manufactured by OxyChem (Venier et al. 2015; Venier et al. 2010).

HCPN was detected in 100% of samples analyzed, ranging from 0.0009–0.07 ng/g ww and a geometric mean of 0.011 ng/g ww. HCPN is an intermediate in the production of chlorinated flame retardants, as well as heptane fungicides, and may also be a result of biotransformation or impurity in the production of these compounds (Sühling et al. 2015). HCPN has been detected in sediments of Lake Ontario at concentrations ranging from 5–41 pg/g dw (0.005–0.041 ng/g dw), as well as lake trout (*Salvelinus namaycush*) and whitefish (*Coregonus clupeiformi*) 0.02–0.32 ng/g lw (0.0012–0.019 ng/g ww, assuming a whole-body fish lipid content default value of 6%)(Schlechtriem et al. 2012; Shen et al. 2014). 1,3-DPMA was also detected in 100% of samples, ranging from 0.00076–0.197 ng/g ww and a geometric mean of 0.016 ng/g ww. 1,3- and 1,5-DPMA are monoadducts, or positional isomers of DP, and are thought to arise from the incomplete reaction of DP or impurities in the DP starting material during its manufacture. (Tomy et al. 2013). Concentrations of 1,3-DPMA were shown to be approximately three times greater than 1,5-DPMA in the recent (surficial) layers of Niagara River Bar sediment profiles. In addition, 1,3-DPMA was measureable in an apex predator, the Lake Ontario lake trout (Sverko et al. 2009). Tomy et al. (2013) found a significant negative relationship between concentration of 1,3-DPMA and trophic level in the Lake Ontario food web, hypothesizing that 1,5-DPMA is more readily metabolized or less bioavailable than 1,3-DPMA due to the position of the less sterically hindered double bond (Tomy et al.

2013). Our results agree with this hypothesis as the 1,5-DPMA was only detected in 15 of 24 samples, ranging from <MDL–0.022 ng/g ww and a geometric mean of 0.0025 ng/g ww.

Similar to DP, Hooker Chemical Co. patented Dec 602, 603, and 604 from the late 1960s to the 1980s for use as flame retardants in polymer products used for electromechanical applications (Shen et al. 2011b). Dec 602 and 604 are likely in commercial use today as they are listed on Canada’s Nondomestic Substances List (CEPA Environmental Registry). Besides this, little information on production and usage of Dec 602, 603, and 604 is available. One of the few studies measuring these compounds in the Great Lakes showed that that Dec 602, 603, and 604 have a greater potential for bioaccumulation than DP in the Lake Ontario environment due to their respective biota-sediment accumulation factors (BSAFs) calculated as (Shen et al. 2014; Shen et al. 2011a):

$$BSAF = \frac{C_B}{C_S}$$

(where CB is the concentration of the contaminant in fish on a wet weight basis and CS is the dry weight sediment concentration) and the octanol-water partition coefficients (Shen et al. 2011a). This study also reported that concentrations in Lake Ontario lake trout were greatest for mirex, followed by Dec 602, which were 50 to 380 times greater than those of DP (Shen et al. 2011a). Although we Dec 602 was detected in 100% of samples, its geometric mean (0.010 ng/g ww, ranging from 0.0017–0.05 ng/g ww) was lesser than total DP. Dec 603 was also detected in 100% of samples, ranging from 0.00036–0.268 ng/g ww and a geometric mean of 0.004 ng/g ww. Dec 604 however, was only detected in one sample in the MMR spatial

region at a concentration of 0.026 ng/g ww. CP is another chlorinated compound developed by the Hooker Chemical Co., also formed by a Diels-Alder reaction and structurally related to DP, Dec 602, 603, and 604. We detected a geometric mean of 0.0027 ng/g ww for CP, ranging from <MDL–0.051 ng/g ww. CP concentrations were found to be greatest near urban areas when measured in tributary sediments of the Great Lakes (Shen et al. 2011b). CP was also frequently detected (> 80%) in fish sampled in the St. Lawrence River, at concentrations up to 73 ng/g lw, strongly suggesting the occurrence of proximate sources (Houde et al. 2014). Declining trends of suspended sediment CP concentrations in the Niagara River from 1980–2006 however, indicate that it is not necessarily an emerging chemical contaminant (Shen et al. 2011a).

Of the three isomers of Dec604 (BrDec604-1a, BrDec604-1b, BrDec604-1c), concentrations of BrDec604-1a and BrDec604-1b were the greatest contributors to Σ Dechlorane concentrations (25 and 24%, respectively). BrDec604-1a was detected in 80% of samples, ranging from <MDL–2.309 ng/g ww. BrDec604-1b was detected in 54% of samples, ranging from <MDL–2.567 ng/g ww. BrDec604-1c also contributed to Σ Dechlorane concentrations (9%), ranging from <MDL–1024.28 ng/g ww and geometric mean of 0.125 ng/g ww. To our knowledge, only one study has detected these compounds in the Great Lakes environment. Shen et al. (2014) only detected the BrDec604-1c analogue, ranging from 0.43–3.8 ng/g lw in lake trout and whitefish from Lake Ontario in 1998 and 1999. Results of this study also suggested that BSAFs increased among the analogues with successively fewer halogens, with BrDec604-1c having the greatest BSAF of 7.1, similar to that calculated for mirex. In

general, concentrations of Dec604 analogues were in agreement with this finding, with the exception of Br₂Cl₂Dec604 which contributed 11% to ΣDechlorane concentrations (range of <MDL–1.509 ng/g ww and geometric mean of 0.231 ng/g ww). Dec604-Cl₄ is also notable because it was detected in 96% of samples (range of <MDL–0.282 ng/g ww and geometric mean of 0.060 ng/g ww).

The BSAFs, log K_{ow} estimates, and greater concentrations in fish of the Dec604 analogues than the parent Dec604 suggests that Br/Cl analogues of Dec604 have a higher potential for bioaccumulation than Dec604 in the environment. There is little information available regarding the production history and current use of Dec604. The relatively abundant number of analogues, and potential to bioaccumulate in the aquatic environment however, highlight the importance of considering impurities and transformation products of halogenated compounds present in the Great Lakes (Shen et al. 2014).

OPs

ΣOP concentrations ranged from 6.19–53.12 ng/g ww, with a geometric mean of 18.14 ng/g ww. These concentrations are about one order of magnitude greater than ΣBDE and ΣDechlorane concentrations and two orders of magnitude greater than ΣNBFR concentrations. ΣOP concentrations were also found to be an average two to three orders of magnitude greater than concentrations of ΣBDEs and two NBFRs, TBB, and TBPH, in atmospheric particle phase samples collected at five sites in the North American Great Lakes basin in 2012 (Salamova et al. 2014). In addition, OP concentrations in feathers of white-tailed eagles (*Haliaeetus albicilla*) (0.95–3000 ng/g) in Norway were also found to be two to three orders of magnitude greater than

all brominated flame retardants. When OPs were measured in plasma of the same nestlings however, only TCIPP and TDCPP were detected at much lower concentrations (median of 0.22 ng/g), in two of the 17 samples. The authors attributed the greater concentrations measured in feathers to inherent passive air sampling during feather growth (Eulaers et al. 2014). Concentrations of Σ OPs were also lower in herring gull eggs collected from colonies in U.S. on Lakes Erie, Huron, Michigan and Superior in 2012 and 2013, with an overall mean concentration of 1.41 ng/g ww, and individual concentrations ranging from non-detected to 13.8 ng/g ww (Su et al. 2015). A previous study also analyzed 15 non-halogenated, chlorinated, or brominated OPs in herring gull eggs from the Great Lakes from 1990–2010, finding concentrations to be consistently low and highly variable between years. In addition, TPP was only detected in eggs collected from two islands in 2008 and 2010, although at concentrations ranging from 2.1–8.2 ng/g ww (Letcher et al. 2011). In our study, TPP contributed the greatest to Σ OP concentrations (25.5%), ranging from 0.57–25.52 ng/g ww and a geometric mean of 3.99 ng/g ww. TPP is one of the most effective additive flame retardants in many polymers and is commonly used in combination with flame retardant mixtures (van der Veen and de Boer 2012). TPP concentrations have been found to be significantly and positively correlated with the seasonally averaged concentrations of the penta-BDE congeners TBB and TBPH, providing support that it was used as a component in the Penta-BDE mixture, and Firemaster 550 (Salamova et al. 2014). TIPPP was the second greatest contributor to Σ OP concentrations (20%), ranging from <MDL–28.60 ng/g ww and a geometric mean of 4.43 ng/g ww. Although this is the first record of measurement of TIPPP in

biota, this finding is particularly interesting, as the number of detections in the atmosphere sampled by Salamova et al. (2014) in the Great Lakes in 2012 were so few that detected concentrations were not included in analyses. The few locations it was detected in the atmosphere however, were near urban areas (Salamova et al. 2014).

TCPP, TPRP, and TnBP contributed similarly to Σ OP concentrations (16%, 14%, and 12%, respectively) in our study. Geometric mean and concentration ranges of TCPP (2.89 ng/g ww, <MDL–15.61 ng/g ww) were greater than concentrations measured in the plasma of white-tailed eagle nestlings in Norway (0.22 ng/g ww, 0.12–0.74 ng/g ww) (Eulaers et al. 2014). TCPP was only detected in 3% of herring gull eggs in 2012 and 2013, whereas TnBP was detected in 50% (Su et al. 2015). The use of TCPP has continued to grow since the mid-1960's as a replacement since tris(2-chloroethyl)phosphate (TCEP) was phased out in the EU, and is currently being phased out in North America (Möller et al. 2011). Salamova et al. (2014) found that atmospheric Σ OP concentrations across the Great Lakes Basin in 2012 were greatest in urban areas (Cleveland and Chicago), and that chlorinated OPs contributed the greatest amount to total concentrations. TCPP was the dominating chlorinated OP at the three urban sites, with concentrations two to seven times greater than the other chlorinated compounds detected. TCPP was also the dominating OP compound of eight analyzed in air samples collected from the German part of the North Sea in 2010 (Möller et al. 2011). TnBP and TPP were found to be the most abundant nonhalogenated OPs in atmospheric concentrations at two remote sites of the Great Lakes. TnBP was unique however, in that concentrations at the remote site of Eagle

Harbor were statistically indistinguishable from the urban areas of Chicago and Cleveland, suggesting that the atmospheric transport potentials of nonhalogenated and halogenated OPs may be similar (Salamova et al. 2014).

Lower OP concentrations observed in Great Lakes herring gull eggs and the plasma of nestling white-tailed eagles in Norway has been proposed to be a result of lower bioavailability, environmental degradation, or metabolic transformation (Eulaers et al. 2014; Su et al. 2015). OP diesters have been found in the plasma of wild herring gulls, providing evidence that metabolism of their respective OP triester precursors occurs *in vivo*. Furthermore, OP diester concentrations were higher than the concentrations of their OP triester flame retardant precursors, with the exception of TPP and its diester degradation product diphenyl phosphate. Other compounds measured in the same study with the greatest concentrations of respective diester products were TDCPP, TBEP, and TEHP (Su et al. 2014a). In our study, TDCPP, TBEP, and TEHP were three of the six compounds least frequently detected (ranging from 4–20%). This suggests that these compounds may be rapidly degraded to their diester products in bald eagles, while compounds with greater detection frequencies and concentrations such as TPP, may be less readily metabolized. Rapid OP metabolism has also been shown in kestrels, as concentrations were undetected in tissues (renal, hepatic) dosed with TBEP, TCEP, TCPP, and TDCPP. Exposure to the four OPs resulted in overall changes in circulating thyroid hormones, particularly in plasma free triiodothyronine, with relative increases of 32–96% at 7 days of exposure. The authors summarize that the physiological and endocrine effects observed may have been due to triester or diester metabolites that were present

despite the rapid degradation of environmentally-relevant OP concentrations (Ferne et al. 2015). Given this, and the greater concentrations of OPs found in our study, further research is required to investigate the metabolic potential and possible chronic effects of long-term OP exposure in wild birds.

While we report only concentrations in nestling bald eagle plasma here, future research on flame retardant compounds in bald eagles will include levels in the paired egg samples from this study as well. This will provide insight into the degree of *in ovo* exposure and adult body burden versus the local food web exposure during the first 6-9 week growth period for nestlings. Food chain studies measuring these compounds in water, sediment, fish, and eggs within a smaller geographic region will also provide information on the propensity of individual compounds to biomagnify through trophic levels.

Tables

Table 3.1 Concentrations (ng/g ww) of polybrominated diphenyl ether (BDE) congeners, and compounds of non-brominated diphenyl ethers (NBFRs), Dechloranes, and organophosphate ester (OPs) flame retardants in bald eagle plasma (*Haliaeetus leucocephalus*) in Michigan (USA) from 1999–2014.

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|----------|--|----------|--|-----------------|--|----------|--|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL |
| ΣBDEs | 4.64 0.477–22.49 24 | ΣNBFRs | 0.179 <MDL–1.249 21 | ΣDechloranes | 1.294 0.101–6.917 24 | ΣOPs | 18.14 6.19–53.11 24 |
| BDE–7 | 0.0013–0.0018 <MDL–0.005 17 | PBBz | – <MDL–0.029 8 | <i>syn</i> -DP | 0.0079–0.0397 <MDL–0.379 15 | TIPRP | 0.4976–0.6404 <MDL–1.33 23 |
| BDE–10 | 0.0007 – <MDL–0.44 4 | TBB | 0.0051 – <MDL–0.174 10 | <i>anti</i> -DP | 0.0025 0.0172–0.0785 <MDL–0.729 13 | TPRP | 0.18 1.76 0.25–13.28 24 |
| BDE–15 | 0.02 0.0119–0.0152 <MDL–0.04 22 | HBCD | 0.02 – <MDL–0.175 3 | Total DP | 0.0078 0.022–0.11 <MDL–1.11 17 | TnBP | 0.25 2.32 0.72–6.78 24 |
| BDE–17 | 0.004 0.008 0.001–0.06 24 | TBPH | 0.13 – <MDL–1.075 2 | HCPN | 0.0025 0.0112 0.0014–0.070 24 | TCEP | 0.73 – ND 0 |
| BDE–28 | 0.0015 0.04 0.006–0.35 24 | DBDPE | 0.16 0.1593–0.2071 <MDL–0.564 13 | 1,3-DPMA | 0.0015 0.0157 0.00075–0.1967 24 | TCPP | NA 2.861–2.8599 <MDL–11.29 22 |
| BDE–30 | 0.0056 – <MDL–0.07 1 | PTBx | 0.11 – ND 0 | 1,5-DPMA | 0.00076 0.00219–0.0047 <MDL–0.02178 15 | TPEP | 0.86 3.99 0.57–20.69 24 |
| BDE–47 | 0.067 1.78 0.14–17.37 24 | PBEB | NA – ND 0 | Kepone | 0.00033 – ND 0 | TDCPP | 0.57 – <MDL–0.189 1 |
| BDE–49 | 0.136 0.15 0.02–2.17 24 | HBB | NA – ND 0 | Mirex | NA 0.1018 0.008–1.892 24 | TPP | 0.19 0.3828–0.4878 <MDL–1.44 22 |
| | 0.022 | | NA | | 0.0081 | | 0.20 |

Table 3.1 Continued

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|----------|--|----------|--|-------------|--|----------|--|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL |
| BDE-66 | 0.0369–0.0569 <MDL–0.23 23 0.0077 | TBE | – ND 0 NA | CP | 0.00359–0.008 <MDL–0.0506 24 0.00046 | TBEP | – <MDL–19.11 4 3.62 |
| BDE-71 | – <MDL–0.04 10 0.0007 | | | Dec601 | – ND 0 NA | EHDP | – <MDL–0.46 7 0.11 |
| BDE-85 | 0.0236–0.052 <MDL–0.26 18 0.0027 | | | Dec602 | 0.0102 0.0017–0.05 24 0.0017 | TEHP | – <MDL–2.94 5 0.35 |
| BDE-99 | 0.90 0.12–4.93 24 0.12 | | | Dec603 | 0.0043 0.0004–0.2681 24 0.00036 | TOTP | – ND 0 NA |
| BDE-100 | 0.50 0.05–2.54 24 0.54 | | | Dec604 | – <MDL–0.0258 1 0.026 | TPTP | – <MDL–0.60 1 0.60 |
| BDE-119 | 0.0438–0.058 <MDL–0.14 19 0.015 | | | Dec604 CB | 0.0471–0.1623 <MDL–1.0947 19 0.0013 | TPPP | 3.465–5.8079 <MDL–28.60 17 1.36 |
| BDE-126 | 0.01 0.006–0.27 24 0.0019 | | | BrDec604-1a | 0.3931–0.670 <MDL–2.3095 19 0.024 | TDMPP | – ND 0 NA |
| BDE-138 | – <MDL–1.20 7 0.59 | | | BrDec604-1b | 0.373–0.699 <MDL–2.567 13 0.057 | TBPP | – <MDL–0.0128 1 0.013 |
| BDE-139 | – <MDL–0.59 1 0.59 | | | BrDec604-1c | 0.1241–0.2539 <MDL–1.0243 14 0.0086 | | |
| BDE-140 | 0.0193–0.0378 <MDL–0.20 20 0.0063 | | | Dec604-2 | – <MDL–0.01248 5 0.0067 | | |

Table 3.1 Continued

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|---------------|--|----------|--|--|--|----------|--|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL |
| BDE-153 | 0.17 0.03–2.00 24 0.026 | | | Dec604-Cl ₄ | 0.0679–0.0967 <MDL–0.2817 19 0.0086 | | |
| BDE-154+BB153 | 0.25 0.03–1.38 24 0.032 | | | Br ₃ Dec604 | – <MDL–0.202 3 0.021 | | |
| BDE-156+169 | – <MDL–0.01 1 0.011 | | | Cl ₁₀ DP | – <MDL–0.0848 1 0.085 | | |
| BDE-180 | – <MDL–0.36 2 0.036 | | | Cl ₁₁ DP | – <MDL–0.001 7 0.00019 | | |
| BDE-183 | – <MDL–0.16 10 0.033 | | | Br ₂ Cl ₂ Dec604 | 0.1845–0.3193 <MDL–1.5092 17 0.0162 | | |
| BDE-184 | 0.0084–0.0350 <MDL–0.32 17 0.0018 | | | | | | |
| BDE-191 | – <MDL–0.10 1 0.10 | | | | | | |
| BDE-196 | – <MDL–0.15 5 0.009 | | | | | | |
| BDE-197 | – <MDL–0.37 11 0.016 | | | | | | |
| BDE-201 | 0.006 0.002–0.16 24 0.0017 | | | | | | |

Table 3.1 Continued

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|----------|--|----------|--|-------------|--|----------|--|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL | Compound | Geometric mean ^a extremes ^b <i>n</i> detected MDL |
| BDE-203 | — <MDL-0.038 4 0.021 | | | | | | |
| BDE-204 | — <MDL-0.002 3 0.0004 | | | | | | |
| BDE-205 | — ND NA 0 | | | | | | |
| BDE-206 | — <MDL-0.03 11 0.0013 | | | | | | |
| BDE-207 | — <MDL-1.12 8 0.0094 | | | | | | |
| BDE-208 | 0.0022-0.0216 <MDL-0.23 12 0.0009 | | | | | | |
| BDE-209 | 0.0257-0.2428 <MDL-2.59 15 0.0086 | | | | | | |

^a — No mean calculated, as more than half of the samples were below the minimum detection limit (MDL); If less than half of the samples were below the MDL, the Kaplan–Meier method was used to estimate the extremes of the mean.

^b — extremes are defined as the minimum and maximum values in the dataset.

Table 3.2. Concentrations (ng/g ww) of polybrominated diphenyl ether (BDE) congeners, and compounds of non-brominated diphenyl ethers (NBFRs), Dechloranes, and organophosphate ester (OPs) flame retardants in bald eagle plasma (*Haliaeetus leucocephalus*) from Lake Superior (LS), Impoundments (IMP), the Menominee River (MMR), and Saginaw Bay (SGB) spatial regions in Michigan (USA) from 1999–2014.

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|-----------|---|------------|---|------------------|---|----------|---|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected |
| ΣBDEs LS | 6.20 ^A 1.19–14.35 6 | ΣNBFRs LS | 0.239 ^A 0.161–0.442 6 | ΣDechloranes LS | 2.59 ^A 0.38–6.92 6 | ΣOPs LS | 16.61 ^A 11.70–28.54 6 |
| ΣBDEs IMP | 1.67 ^A .48–3.29 6 | ΣNBFRs IMP | 0.0802–0.233 ^A <MDL–0.425 4 | ΣDechloranes IMP | 0.64 ^A 0.43–1.25 6 | ΣOPs IMP | 18.02 ^A 8.84–49.72 6 |
| ΣBDEs MMR | 6.69 ^A 1.01–22.49 6 | ΣNBFRs MMR | 0.112–0.282 ^A <MDL–0.564 5 | ΣDechloranes MMR | 1.08 ^A 0.34–2.52 6 | ΣOPs MMR | 20.60 ^A 6.19–53.12 6 |
| ΣBDEs SGB | 6.72 ^A 2.00–18.93 6 | ΣNBFRs SGB | 0.232 ^A 0.108–1.25 6 | ΣDechloranes SGB | 1.55 ^A 0.10–6.81 6 | ΣOPS SGB | 17.56 ^A 7.18–49.81 6 |

^a – No mean calculated, as more than half of the samples were below the minimum detection limit (MDL); If less than half of the samples were below the MDL, the Kaplan–Meier method was used to estimate the extremes of the mean. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), or a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction; ND = Not detected.

^b – extremes are defined as the minimum and maximum values in the dataset.

Table 3.3 Concentrations (ng/g ww) of polybrominated diphenyl ether (BDE) congeners, and compounds of non-brominated diphenyl ethers (NBFRs), Dechloranes, and organophosphate ester (OPs) flame retardants in bald eagle plasma (*Haliaeetus leucocephalus*) from Inland (IN) and Great Lakes (GL) breeding areas in Michigan (USA) from 1999–2014.

| BDEs | | NBFRs | | Dechloranes | | OPs | |
|----------|---|-----------|---|-----------------|---|----------|---|
| Congener | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected | Compound | Geometric mean ^a extremes ^b <i>n</i> detected |
| ΣBDEs IN | 3.215 ^A 0.477–22.49 13 | ΣNBFRs IN | 0.118–0.22 ^A <MDL–0.564 13 | ΣDechloranes IN | 0.711 ^B 0.101–2.52 13 | ΣOPs IN | 18.73 ^A 6.19–53.12 13 |
| ΣBDEs GL | 7.179 ^A 1.199–18.934 11 | ΣNBFRs GL | 2.63 ^A 0.389–6.92 11 | ΣDechloranes GL | 2.63 ^A 0.389–6.92 11 | ΣOPs GL | 17.46 ^A 7.176–49.18 11 |

^a – No mean calculated, as more than half of the samples were below the minimum detection limit (MDL); If less than half of the samples were below the MDL, the Kaplan–Meier method was used to estimate the extremes of the mean. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), or a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction; ND = Not detected.

^b – extremes are defined as the minimum and maximum values in the dataset.

Table 3.4 Concentrations (ng/g ww) of Dechlorane compounds in bald eagle plasma (*Haliaeetus leucocephalus*) in Michigan (USA) from 1999–2014.

| | Inland (n = 13) | Great Lakes (n = 11) |
|-----------------|---|---|
| | Geometric mean ^a extremes ^b <i>n</i> detected | Geometric mean ^a extremes ^b <i>n</i> detected |
| <i>syn</i> -DP | 0.0029–0.0622 ^A <MDL–0.379 8 | 0.009–0.02 ^A <MDL–0.0629 7 |
| <i>anti</i> -DP | 0.0066–0.123 ^A <MDL–0.729 7 | 0.0167–0.0393 ^A <MDL–0.122 6 |
| Total DP | 0.008–0.179 ^A <MDL–1.108 10 | 0.0215–0.056 ^A <MDL–0.185 7 |
| HCPN | 0.0059 ^B 0.0015–0.029 13 | 0.024 ^A 0.0032–0.07 11 |
| 1,3-DPMA | 0.0057 ^B 0.0008–0.0202 13 | 0.053 ^A 0.013–0.197 11 |
| 1,5-DPMA | 0.00324–0.0077 ^A <MDL–0.022 9 | 0.00097–0.00154 ^A <MDL–0.0036 6 |
| Kepone | – ND 0 | – ND 0 |
| Mirex | 0.061 ^B 0.008–0.527 13 | 0.366 ^A 0.043–1.89 11 |
| CP | 0.0013–0.0028 ^B <MDL–0.010 11 | 0.0049 ^A 0.0007–0.051 11 |
| Dec601 | – ND 0 | – ND 0 |
| Dec602 | 0.005 ^B 0.0017–0.015 13 | 0.0237 ^A 0.0085–0.05 11 |
| Dec603 | 0.0022 ^B 0.0004–0.057 13 | 0.0096 ^A 0.0015–0.27 11 |
| Dec604 | – <MDL–0.026 1 | – ND 0 |
| Dec604 CB | 0.0024–0.0097 ^A <MDL–0.048 8 | 0.0135 ^A 0.0013–1.095 11 |
| BrDec604-1a | 0.066–0.124 ^B <MDL–0.324 8 | 0.756 ^A 0.05–2.31 11 |

Table 3.4 Continued

| | Inland (n = 13) | Great Lakes (n = 11) |
|--|---|---|
| | Geometric mean ^a extremes ^b <i>n</i> detected | Geometric mean ^a extremes ^b <i>n</i> detected |
| BrDec604-1b | 0.209–0.424 <MDL–1.33 8 | – <MDL–2.57 5 |
| BrDec604-1c | – <MDL–0.105 6 | 0.262–0.499 <MDL–1.024 8 |
| Dec604-2 | – <MDL–0.012 2 | – <MDL–0.01 3 |
| Dec604-Cl ₄ | 0.0319–0.0504 ^B <MDL–0.118 12 | 0.114 ^A – 11 |
| Br ₃ Dec604 | – ND 0 | – <MDL–0.202 3 |
| Cl ₁₀ DP | – ND 0 | – <MDL–0.085 1 |
| Cl ₁₁ DP | – <MDL–0.001 2 | – <MDL–0.00078 5 |
| Br ₂ Cl ₂ Dec604 | 0.134–0.20 ^A <MDL–0.44 10 | 0.243–0.516 ^A <MDL–1.51 7 |

^a – No mean calculated, as more than half of the samples were below the minimum detection limit (MDL); If less than half of the samples were below the MDL, the Kaplan–Meier method was used to estimate the extremes of the mean. Means with different capital letters are significantly different by Tukey's HSD method of multiple comparisons ($p < 0.05$), or a generalized Wilcoxon non-parametric test followed by pairwise comparisons using a Bonferroni correction; ND = Not detected.

^b – extremes are defined as the minimum and maximum values in the dataset.

Figures

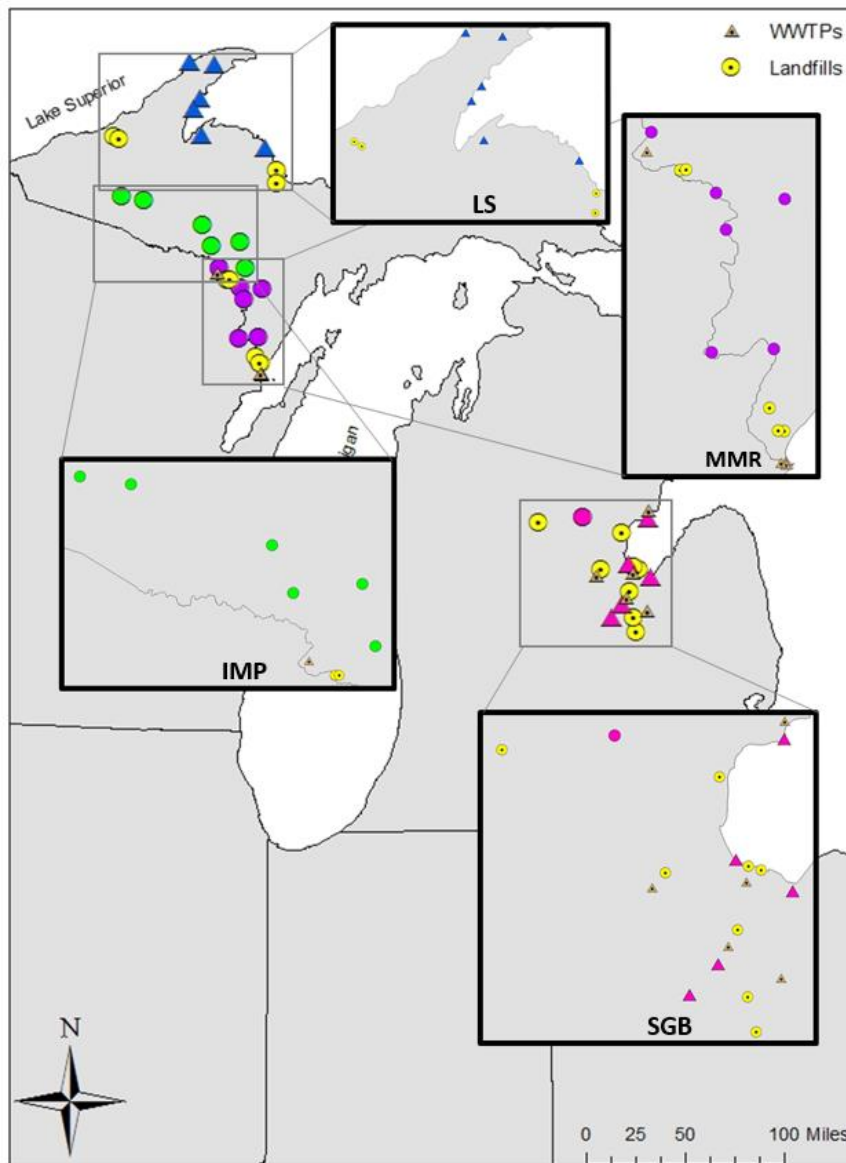


Figure 3.1 Spatial regions Lake Superior Great Lake (LS), Impoundments (IMP), Menominee River (MMR), and Saginaw Bay (SGB), and proximate wastewater Treatment Plants (WWTPs) and Landfills in which bald eagle (*Haliaeetus leucocephalus*) plasma samples were collected between 2000 and 2012 for flame retardant analysis. Great Lakes breeding areas are triangular and Inland breeding areas are circular.

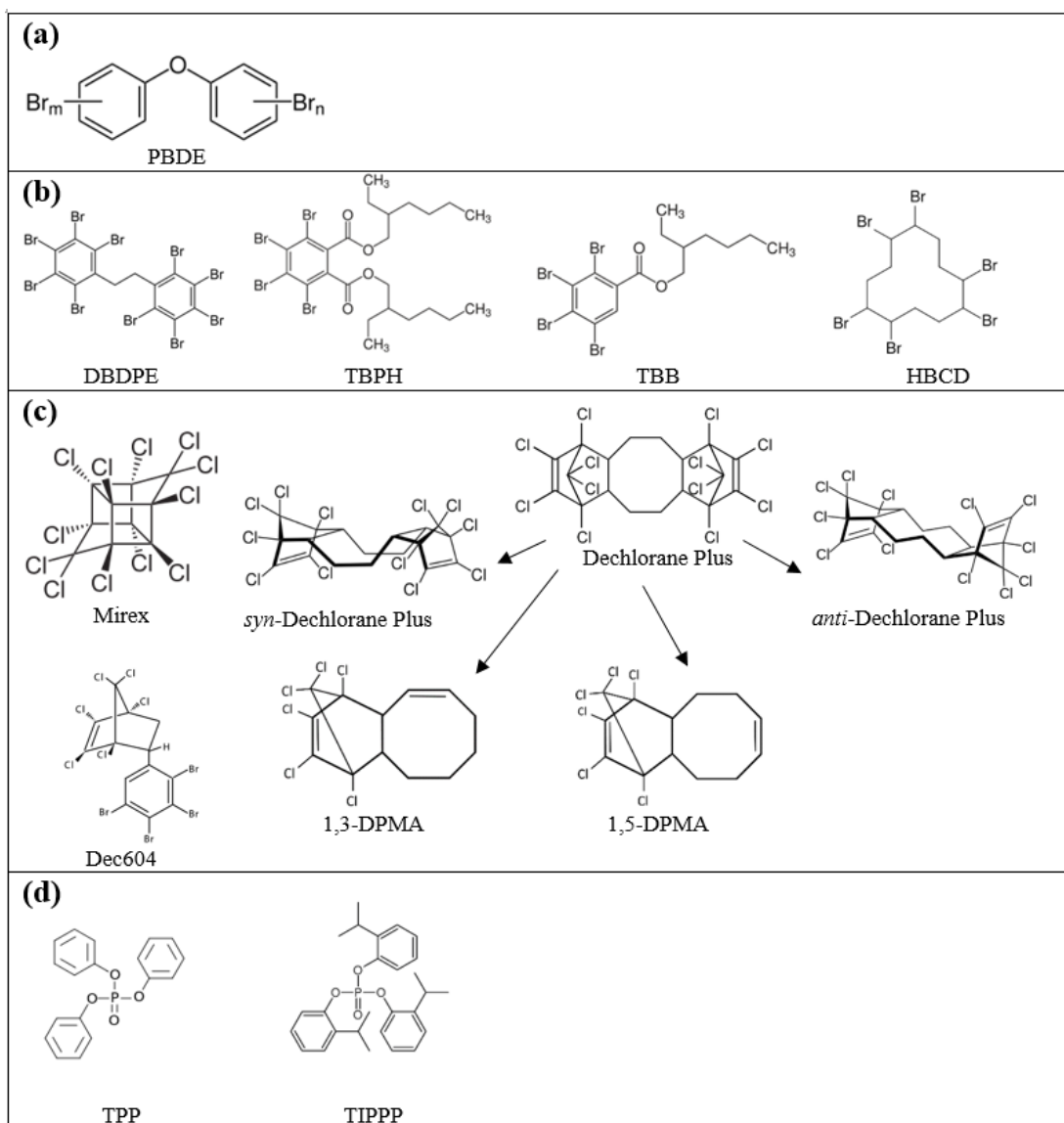


Figure 3.2 Chemical structures of (a) polybrominated diphenyl ethers (BDEs), (b) non- polybrominated diphenyl ethers flame retardants (NBFRs); decabromodiphenylethane (DBDPE), bis(2-ethylhexyl)-3,4,5,6-tetrabromo-phthalate (TBPH), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), Hexabromocyclododecane (HBCD), (c) Dechloranes; Mirex, Dechlorane Plus (DP): *syn*- and *anti*- isomers, 1,3-Dechlorane Plus-monoadduct (1,3-DPMA), 1,5-Dechlorane Plus-monoadduct (1,3-DPMA), hexachloro(phenyl)-norbornene (HCPN), Dechlorane 604 (Dec604), and (d) organophosphate esters (OPs); triphenyl phosphate (TPP), and tris-(2-isopropylphenyl) (TIPPP).

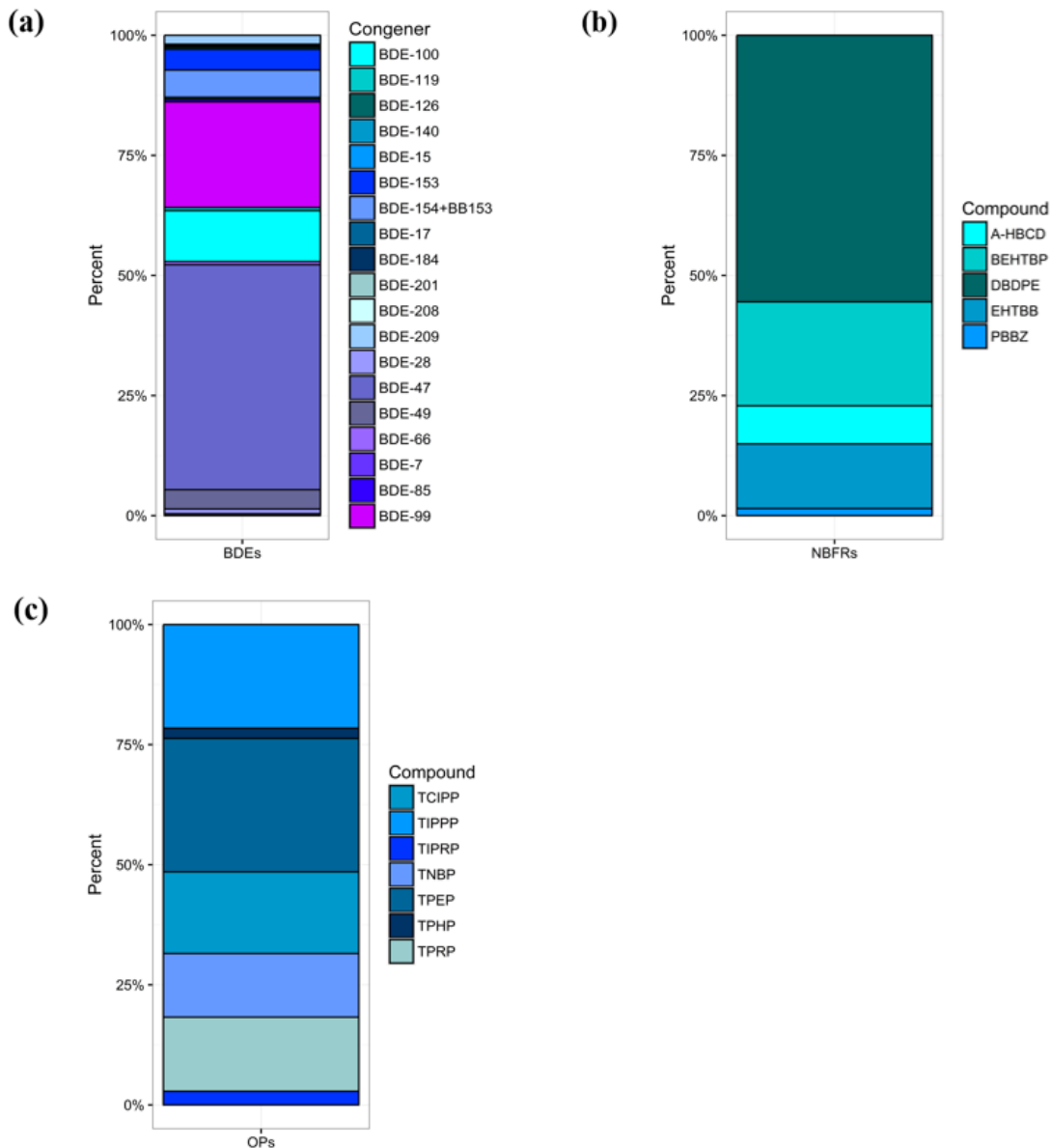


Figure 3.3 Percent contribution of (a) polybrominated diphenyl ether (BDE) congeners, (b) non-PBDE brominated flame retardant (NBFR) compounds, and (c) organophosphate ester (OP) compounds in Michigan (USA) bald eagles (*Haliaeetus leucocephalus*) between 2000 and 2012. Only congeners in which less than 50% of the samples were below the minimum detection limit are shown.

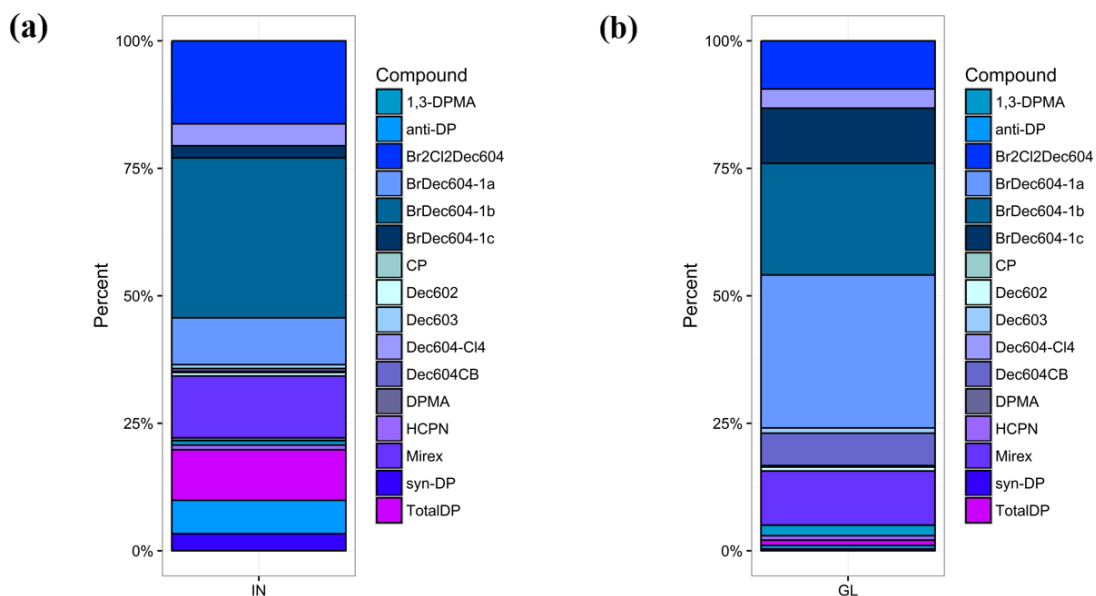


Figure 3.4 Percent contribution of Dechlorane compounds in (a) Inland (IN) and (b) Great Lakes (GL) breeding areas in Michigan (USA) bald eagles (*Haliaeetus leucocephalus*) between 2000 and 2012. Only congeners in which less than 50% of the samples were below the minimum detection limit are shown.

Chapter 4: Historic and Emerging Sources of Mortality in Bald Eagles in Michigan, 1987-2011

Introduction

The bald eagle (*Haliaeetus leucocephalus*) is a large fish-eating bird of prey with an extensive breeding and wintering range located mainly in the contiguous United States and southern Canada, including coastal areas of northern Canada and Alaska (Buehler 2000). Although typically a fish-eating bird, the bald eagle is an adaptable forager and will scavenge and pirate a variety of other prey species including mammalian, avian, reptilian, and carrion (Buehler 2000). Populations of bald eagles substantially declined in the mid-twentieth century mostly due to human persecution and the release of organochlorine pesticides and polychlorinated biphenyls. Only after the Endangered Species Act of 1973 and the ban of numerous organochlorine compounds by the Environmental Protection Agency in the 1970's have bald eagle populations recovered to the historic levels (Buehler 2000). However, the recovery of bald eagle populations has been accompanied by new and emerging threats of mortality beyond those eliminated or reduced by the legislation of the 1970's and 80's (U.S. Fish and Wildlife Service 2007).

Anthropogenic sources of mortality, such as vehicular trauma, have been shown to be a significant threat to bald eagles (Harris and Sleeman 2007; Russell and Franson 2014). Predatory and scavenging birds, like bald eagles, often rely on roadside carrion as a main prey base making them particularly vulnerable to trauma-based mortality because of vehicular collision (Kelly et al. 2014; Russell and Franson

2014; Wood et al. 1990). Another common anthropogenic mortality source for bald eagles is poisoning, specifically lead toxicosis (Harris and Sleeman 2007; Hunt et al. 2006; Johnson et al. 2013; Kelly et al. 2014; Pagel et al. 2012; Stauber et al. 2010; Warner et al. 2014). Historically, lead toxicosis in bald eagles was a direct result of the ingestion of lead shot from dead or wounded waterfowl, and was a major factor leading to the ban of lead shot for waterfowl hunting in 1991 (Friend et al. 2009; Kendall et al. 1996). Recently, research has linked lead toxicosis in bald eagles to ingested lead fragments embedded in tissues or offal of lost or discarded upland and large game animals (Stauber et al. 2010; Warner et al. 2014). Amplifying this exposure source is the high incidence of lead bullet fissuring upon impact, resulting in fragments of irregular shapes and greater surface areas that dissolve more easily in stomach acids, increasing metal retention and ultimately the magnitude of exposure (Fisher et al. 2006; Hunt et al. 2009; Scheuhammer and Templeton 1998; Warner et al. 2014). In addition, the high density and small particle size of fragments increases the probability of ingestion by bald eagles and scavengers alike (Haig et al. 2014).

As bald eagle populations recover, estimates of the cause and number of mortality events associated with these emerging sources will become important population vital rates when trying to assess population turnover, population stability, and the vulnerability of different age groups or sexes within different populations (Newton 1979). Defining major sources of mortality, or increases in a certain source over time, provides managers important information to develop management plans and mitigation efforts. Our main goals of this study are to determine the major sources of mortality within the bald eagle population in Michigan, and identify any

trends in these sources of mortality. We also aim to investigate life history traits, in relation to human-influenced factors, that predispose bald eagles to the major sources of mortality.

Methods

The Michigan bald eagle population monitoring program (MBEPMP) began in cooperation with the Continental Bald Eagle Project of the National Audubon Society in 1961 (Postupalsky 1985). The program aimed to monitor and assess bald eagle populations through annual aerial surveys, and document the population recovery from a low of 52 occupied breeding areas in 1961 to approximately 656 in 2011 (Simon 2013). Before 1987, the United States Fish and Wildlife Service (USFWS), through the National Wildlife Health Center as part of the MBEPMP, attempted to determine the cause of death for all bald eagle carcasses recovered across the continental U.S (U.S. Fish and Wildlife Service 1983). Upon cessation of this program, the Michigan Department of Natural Resources (MDNR), through its Wildlife Disease Laboratory, began conducting necropsies on all carcasses collected in Michigan. During this same period, MDNR in cooperation with the Veterinary Clinical Center at Michigan State University also collected, treated, and if possible released grounded bald eagles. In total, the bald eagle recovery program implemented by MDNR recovered 1,001 bald eagles. Less than 5% were recovered alive, treated, and released or placed in captivity. Although the sample is sufficiently large it does not represent a random sample; some “Causes of Death (COD)” may be over-represented, such as vehicular trauma due to direct human reporting and high

visibility of carcasses along roadsides. In contrast, the number of eagles recovered due to lead poisoning may be under-represented.

We determined the cause of mortality COD for every eagle carcass recovered from 1987 to 2011 in the state of Michigan using a generalized examination by systems necropsy. The necropsy results determined a single primary diagnosis, or COD, for each recovered eagle. We divided the 1,001 primary diagnoses into seven general categories: no diagnosis, trauma, poisoning, conditioning (including starvation, malnutrition, or dehydration), drowning, deformity, and diseases and infectious agents. We further divided trauma and poisoning CODs to determine leading COD within these primary diagnoses (ie. vehicular trauma, lead poisoning, etc.). We also assigned each recovered eagle an approximate month of recovery.

We diagnosed trauma CODs based on the presence of bone fractures and hemorrhaging in recovered eagles. Histories provided with the eagle also gave insight into the specific trauma event (example: found along roadside or under a powerline). We sampled and tested livers from every carcass for lead levels. We considered lead levels at or above 5 mg/kg wet weight (ww) to be significant and indicative of lead poisoning. When lead levels were above this level, lead poisoning was considered the primary COD. For example, if the history of a recovered carcass indicated that it was found along a roadside and the necropsy results agreed (such as evidence of broken bones), the proximate COD would be vehicular trauma. Upon analysis of lead levels exceeding 5mg/kg ww however, the ultimate COD was considered lead poisoning. Hypostatic congestion was the only major post-mortem change. This was not considered to affect necropsy diagnoses however, as it is normally observed in the

viscera, not the musculature. We tested carcasses recovered in late summer or early fall for *Clostridium botulinum* type C and E using a mouse bioassay described by Quortrup and Sudheimer (1943). Only recently collected carcasses, in fair condition were sampled to protect against false positive results due to postmortem production of toxicants. We tested all eagles recovered from landfills for pentobarbital and phenytoin poisoning, or when the histories provided suggested a poisoning event. We considered the presence of pentobarbital or phenytoin in a recovered eagle to be significant to cause poisoning. Organochlorines, mercury (acute and chronic), iron (acute and chronic), and selenium were also COD poisoning diagnoses. They were not included in analyses however, because zero recovered eagles were attributed to these diagnoses.

Statistical Methods

We used Generalized Linear Mixed Effects Models (GLMM) to determine differences between bald eagle CODs from 1987 to 2011. We modeled all mortalities as our response variable with year as a random effect on the intercept to account for correlation. This blocked the data by year to determine differences between diagnoses within the year. We included the diagnosis of the COD (i.e. trauma, poisoning, etc.) as our explanatory variables. We then compared the mean differences between β estimates for each diagnosis using the t-distribution with 168 degrees of freedom. We used a similar model formulation of the GLMM to compare the count of each specific diagnoses (i.e. vehicular collision, and lead poisoning.) within Trauma and Poisoning CODs separately. For trauma COD analyses, 178 “unspecified trauma” cases were removed as this diagnosis was not informative. We used the mean difference between

β estimates and the t -distribution to determine different groups of diagnoses within each COD. Because GLMMs do not require data to be normally distributed, we used the raw, non-transformed data and a Poisson error distribution. We performed all statistical analysis using R 3.2.3 (R Development Core Team 2015).

We determined if relationships existed between total COD, trauma COD and poisoning COD to the total numbers of occupied breeding areas over the study period, 1987-2011, using Pearson's correlation coefficient. We also examined the cumulative yearly COD, total COD, vehicular trauma and lead poisoning, using generalized linear models (GLM) to determine if trends in each COD were increasing and if that trend was changing over time. Our GLMs were fit using the Poisson distribution and a log link function. We used the annual count of CODs as the dependent variable in formulas. We fit four models to the total annual count of dead or grounded bald eagles, the annual count of dead or grounded bald eagles due to vehicular trauma, and the annual count of dead or grounded bald eagles due to lead poisoning. The models consisted of a null (intercept only model) to compare other models with, a linear trend model, a quadratic model, and an exponential model, all with year as the independent predictor variable. We compared these models using Akaike Information Criteria corrected for small sample size (AICc) (Burnham and Anderson 2004). When we identified more than one top model, meaning models within 2 AICc of the lowest AICc model score, we tested for significant differences between those models using an Analysis of Variance with a Chi-square test statistic. We graphically overlaid the back-transformed, top model and its 95% confidence intervals over the raw count data by year to illustrate the trends in the data.

Results

Our analyses indicate trauma (n= 595) was the greatest factor leading to the recovery of dead or grounded bald eagles throughout the 25 year period ($P < 0.0001$; Table 4.1). Our comparisons within the trauma category COD indicates that vehicular trauma (n=268) was the greatest factor leading to the recovery of dead or grounded bald eagles ($P < 0.0001$; Table 4.2), occurring primarily in females during the months of September, October, November, and January (Fig. 4.1). Poisoning (n= 106) was the second leading definitive COD (Table 4.1). Comparisons within the poisoning category COD indicated that lead poisoning (n= 99) was the greatest factor leading to the death or injury of recovered bald eagles ($P < 0.0001$; Table 4.3), occurring primarily in females during the months of March, April, and May (Figure 4.1).

Our correlation analysis indicated that the number of active breeding areas was significantly correlated with the total count of dead or grounded eagles ($\rho = 0.96$, $P \leq 0.001$), vehicular trauma ($\rho = 0.88$, $P \leq 0.001$), and lead poisoning ($\rho = 0.76$, $P \leq 0.001$; Figure 4.2). Our trend model analysis for total dead or grounded events indicated that a linear trend and a quadratic model (Table 4.4) best explained the trend in count of annual total dead or grounded bald eagles. When we tested the difference between these models we found they were not significantly different (p-value = 0.117) and chose the quadratic model for comparisons because it had the same model structure as other top models (Figure 4.3A). The trend model analysis for dead or grounded due to vehicular trauma and lead poisoning both indicated the best trend model was the quadratic model (Table 4.4; Figures 4.3B and 4.3C).

Discussion

As bald eagle populations increase and optimal habitats and food sources become depleted, they are likely becoming more dependent on alternative food sources such as carrion during winter months (Stauber et al. 2010; Warner et al. 2014). The observed increase is likely due to the high availability of deer carcasses, and carrion along roadways following deer-vehicle collisions (DVCs) (Sudharsan et al. 2006). In Michigan, 53,592 DVCs were reported in 2011 alone, with the majority of these collisions occurring in October and November (Michigan Department of State Police 2011). The breeding behavior of deer observed in late October and early November, along with the fall hunting season, is likely to blame for the high number of DVCs during the same period (Etter et al. 2002; Sudharsan et al. 2006). Consequently, the majority of bald eagles with COD attributed to vehicular trauma were collected in October. The second greatest incidence of vehicular-collision mortality occurred in January. This may be due to a functional response in changes in availability of aquatic food sources to terrestrial food sources as during winter months as similarly proposed by Grubb and Lopez (2000), Stauber et al. (2010), Nadjafzadeh et al. (2013), and Warner et al. (2014). We also found that females were responsible for the majority of vehicular collision mortality events. We hypothesize that the greater body size of female eagles results in less maneuverability and a longer take off time in the event of oncoming traffic, possibly increasing the number of females killed by vehicular trauma. The female majority in vehicular trauma mortalities may also be due to dominant females out-competing smaller males for carrion when food

sources are limited (Franson and Russell 2014). The high number of vehicular trauma mortalities may be exacerbated when roadside conditions prevent escape routes from oncoming traffic and limit flight paths to traffic lanes.

In Michigan, poisoning was the second greatest definitive cause of bald eagle death or grounding, with lead toxicosis being the primary COD. Other research indicates a dietary shift to more terrestrial prey, especially lost or discarded upland game, during winter months due to frozen water bodies limiting aquatic food sources (Nadjafzadeh et al. 2013). We suspect the reliance on terrestrial prey sources, and the magnitude of increased mortality associated with these sources, is a density dependent effect in Michigan. Our results indicate a correlation exists between lead poisoning and bald eagle breeding density. In addition, the occurrence of lead poisonings has increased at a rate greater than the total number of dead or grounded eagles, up to five times faster towards the end of the study period as indicated by our quadratic model. This suggests that as populations have increased, so has their reliance on the terrestrial prey sources and the subsequent lead poisoning events from these sources. This density dependent reliance on non-preferred food sources has also been suggested by Krüger and Lindström (2001) and Ferrer et al. (2006) who found that as raptor breeding densities increase, inexperienced or non-dominant birds are forced to settle in lower quality habitat. Eagles occupying lower-quality habitat that lacks access to open water, as well as winter migrant eagles, may switch to a less preferred food source such as upland game. For example, changes in natural prey-suitable habitat caused the Verreauxs' eagle (*Aquila verreauxii*) to switch from a favored diet of rock hyrax (*Procavia capensis*), a mammal, to less characteristic avian prey species of

helmeted guineafowl (*Numida meleagris*), francolins (*Francolinus spp.*), and supplemented food (Symes and Kruger 2012). The impact of lead poisoning as a source of mortality may also be amplified by the non-random sample in our study, which likely underestimates the number of eagles dying of lead poisoning.

Lead toxicosis has altered population dynamics of recovering raptor species such as the California condor (*Gymnogyps californianus*), the white-tailed eagle (*Haliaeetus albicilla*), and the Steller's sea eagle (*Haliaeetus pelagicus*) (Church et al. 2006; Johnson et al. 2013; Kim et al. 1999). In addition to birds of prey, corvids and terrestrial carnivores are negatively impacted by spent lead ammunition in game animal remains (Nadjafzadeh et al. 2013). While lead ammunition is no longer used in the hunting of waterfowl, over 69,000 metric tons of lead-based ammunition produced in the United States were utilized in 2012 (Bellinger et al. 2013). Lead ammunition continues to be used in Michigan for hunting upland small game and white-tailed deer. Our results agree with previous studies that have shown that the majority of lead-poisoned bald eagles were found between the months of January and April, when preserved carcasses, containing lead ammunition, become a secondary or supplemental food source (Pattee et al. 1990). Deer that were wounded during hunting season may become stressed by decreased food availability and heavy snows, providing carcasses in the late winter and early spring as well (Neumann 2009). Pattee et al. (1990) suggested that the continued observation of lead poisoning in the months following the fall hunting season could be due to a refractory period between ingestion and observed adverse toxicological effects. For example, death of bald eagles following ingestion of lead shot has been shown to take anywhere from 10 to

133 days (Pattee et al. 1981). We also see a greater number of adult female bald eagles recovered due to lead poisoning. This is consistent with a previous nation-wide study on lead poisoning in bald eagles in which 47% of lead-poisoned birds were adult females but that this age-sex group only comprised about 25% of the total population, demonstrating a specific susceptibility to lead poisoning (U.S. Fish and Wildlife Service 1986). In addition, the total number of female eagles (n= 298) diagnosed with lead poisoning that were admitted to The National Wildlife Health Center from 1975-2013 was also greater than the number of male eagles (n= 175), adults, and juveniles combined. Similar to vehicular trauma COD, this is likely to be a result of dominant females having priority of carrion over smaller males (Franson and Russell 2014). The observed increase in female COD during the months of March, April, and May seen in our data may be due to mobilization of lead from bone for egg laying during breeding periods or increased concentrations as body weight decreases due to preferential feeding of the young, although further research is needed to explore these possible processes. Because bald eagles are a long-lived species with low recruitment rates, vulnerability of adult females to lead poisoning and vehicular trauma could affect the productivity of a population that strongly depends on the survival and reproduction of adult birds (Newton 1979). Lead toxicity has also caused reproductive sterility, a reduced number of offspring, and both morbidity and mortality in neonates, further depressing population productivity (Kendall et al. 1996).

Fishing tackle is also a source of lead exposure when eagles consume fish or other birds that have ingested lead sinkers (Haig et al. 2014; Lewin et al. 2006;

Scheuhammer et al. 2003). The greatest number of recovered dead or grounded bald eagles observed during the spring months may correlate when lost fish, with the line and sinker still attached that have died during winter, become available during ice-out in Michigan. This however is beyond the scope of this paper and requires further investigation.

Clostridium botulinum type E is one COD that has increased within the last five years. Six of the total 22 recovered dead or grounded eagles affected by Disease or Infectious Agents from 2007-2011 were due to type E botulism. These natural botulism outbreaks occur cyclically (annually from July through November) during years of low mean annual water and lake levels, as well as warmer mean surface water temperatures (Lafrancois et al. 2011). Fish, particularly the invasive round goby (*Neogobius melanostomus*), walleye (*Stizostedion vitreum*), and yellow perch (*Perca flavescens*) have been reported as possible significant vectors to various fish-eating birds (Yule et al. 2006). The incidence of type E botulism in bald eagles will likely continue to increase with rising lake temperatures and lower water levels according to Great Lakes climate change predictions (Lafrancois et al. 2011). Another uncommonly known COD shown in our data is poisoning from domestic pets and farm animals euthanized by barbiturate solutions (n= 6), particularly those scavenging in landfills. A total of 29 bald eagles were poisoned after feeding on a cow that had been euthanized in British Columbia, showing the widespread negative effects from a single exposed carcass (Langelier 1993). Secondary sodium pentobarbital poisoning may weaken eagles, causing primary CODs to be caused by blunt trauma (wandering into traffic or falling from perches), predation, drowning, fatal mobbing attacks by

other species, power line collision, or electrocution (Friend and Franson 1999; Krueger and Krueger 2003). Veterinary practitioners and animal shelters may reduce barbiturate poisonings in scavengers by wrapping carcasses euthanized with barbiturate solutions before transportation to landfills. Like lead poisoning, CODs due to type E botulism and barbiturate solutions may be under-represented due to the decreased likelihood that affected eagles will be discovered.

Management Implications

To reduce or mitigate some of these sources of mortality we recommend the removal of deer carcasses and carrion from the roadway through the Bald and Golden Eagle Protection Act, as administered by the U.S. Fish and Wildlife Service. It would also be advisable for County Road Commissions and the Department of Transportation to voluntarily attempt efforts to mitigate this source of incidental take from their roadways. Secondly, a transition to copper or copper-zinc alloy bullets may also ameliorate lead toxicosis mortality as a tangible, yet equally lethal (Trinogga et al. 2013), alternative to lead-based bullets. In addition to not posing a toxic threat if ingested (Thomas 2013), copper bullets are less likely to fragment upon impact, reducing the likelihood of ingestion and increasing the ease of regurgitation.

Tables

Table 4.1 Generalized linear mixed model results for bald eagle (*Haliaeetus leucocephalus*) cause of death or grounding (COD) in Michigan, 1987-2011.

| COD | Number recovered ^a |
|--|-------------------------------|
| Trauma | 595 A |
| No Diagnosis | 161 B |
| Poisoning | 106 C |
| Starvation, Malnutrition, or Dehydration | 56 D |
| Disease | 55 D |
| Drowning | 15 E |
| Deformity | 13 E |

^aMeans with the same letters are not different ($p > 05$)

Table 4.2 Generalized linear mixed model results for bald eagle (*Haliaeetus leucocephalus*) cause of death or grounding (COD) due to trauma in Michigan, 1987-2011.

| Trauma COD | Number recovered ^a |
|------------------------------|-------------------------------|
| Vehicle | 268 A |
| Gunshot | 45 B |
| Electrocution | 41 B |
| Possible Vehicle | 21 B C |
| Predator or Eagle Aggression | 10 C |
| Nest Accident | 10 C |
| Steel Trap | 8 C |
| Powerline or Pole Collision | 7 C |
| Tower Collision | 3 C |
| Airplane | 2 C |
| Golf Ball | 1 C |
| Fishing Gear | 1 C |

^aMeans with the same letters are not different ($p > 0.05$)

Table 4.3 Generalized linear mixed model results for bald eagle (*Haliaeetus leucocephalus*) cause of death or grounding (COD) due to poisoning in Michigan, 1987-2011.

| Poisoning COD | Number recovered ^a |
|----------------------------|-------------------------------|
| Lead | 99 A |
| Pentobarbital or Phenytoin | 6 B |
| Oil | 1 B |

^aMeans with the same letters are not different ($p > 0.05$)

Table 4.4 Trend model selection for total dead or grounded bald eagles (*Haliaeetus leucocephalus*), dead or grounded bald eagles due to vehicular trauma, and dead or grounded bald eagles due to lead poisoning in Michigan from 1987 to 2011. The column heading k defines the number of model parameters estimated, AIC_c is Akaike Information Criteria corrected for small sample size, ΔAIC_c is the difference in AIC_c score from the lowest ranked model, and ω_i is the model weights.

| Mortality Source and Models | k | AIC_c | ΔAIC_c | ω_i |
|--|-----|---------|----------------|------------|
| Total Mortalities | | | | |
| Linear | 2 | 149.90 | 0.00 | 0.60 |
| Quadratic ($0.77 \cdot \text{year}^2 + 15.68 \cdot \text{year} + 28.18$) | 3 | 150.70 | 0.78 | 0.40 |
| Exponential | 2 | 318.20 | 168.28 | 0.00 |
| Null | 1 | 354.60 | 204.74 | 0.00 |
| Vehicular Trauma Mortalities | | | | |
| Quadratic ($0.3 \cdot \text{year}^2 + 133.14 \cdot \text{year} + 7.01$) | 3 | 148.10 | 0.00 | 0.97 |
| Linear | 2 | 155.00 | 6.87 | 0.03 |
| Exponential | 2 | 260.70 | 112.60 | 0.00 |
| Null | 1 | 287.30 | 139.17 | 0.00 |
| Lead Toxicosis Mortalities | | | | |
| Quadratic ($5.06 \cdot \text{year}^2 + 39.62 \cdot \text{year} + 2.7$) | 3 | 106.50 | 0.00 | 0.94 |
| Linear | 2 | 111.90 | 5.44 | 0.06 |
| Exponential | 2 | 158.10 | 51.64 | 0.00 |
| Null | 1 | 183.20 | 76.67 | 0.00 |

Figures

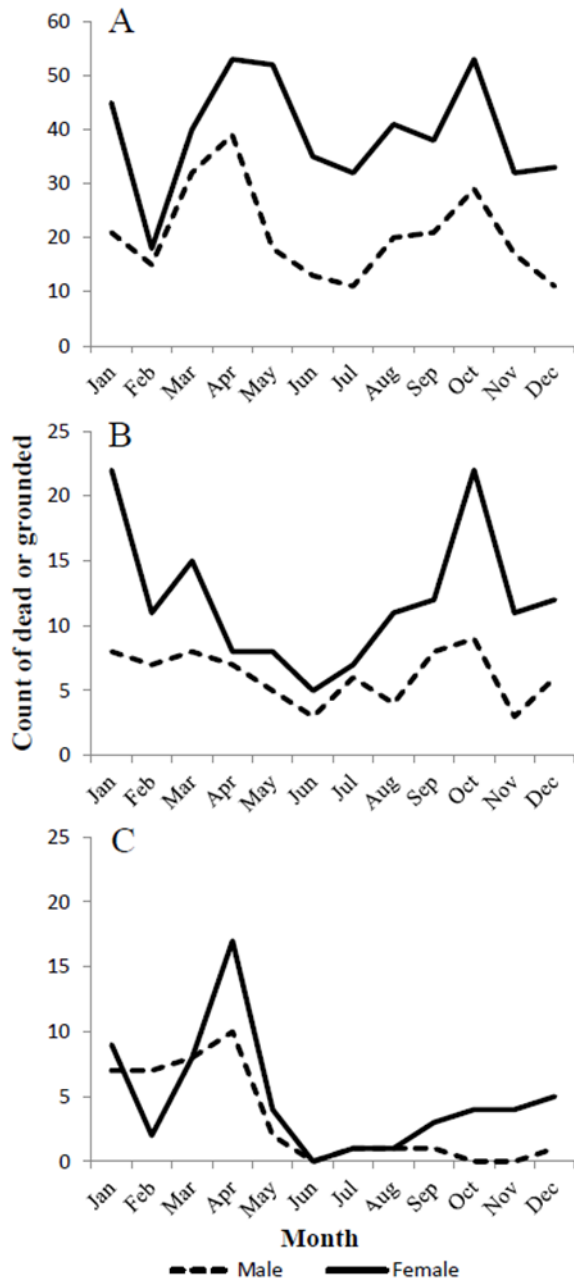


Figure 4.1 Total count of dead or grounded bald eagles (*Haliaeetus leucocephalus*)^A, count of dead or grounded bald eagles due to vehicular trauma^B, and count of dead or grounded bald eagles due to lead poisoning^C for males and females by month in Michigan from 1987 to 2011.

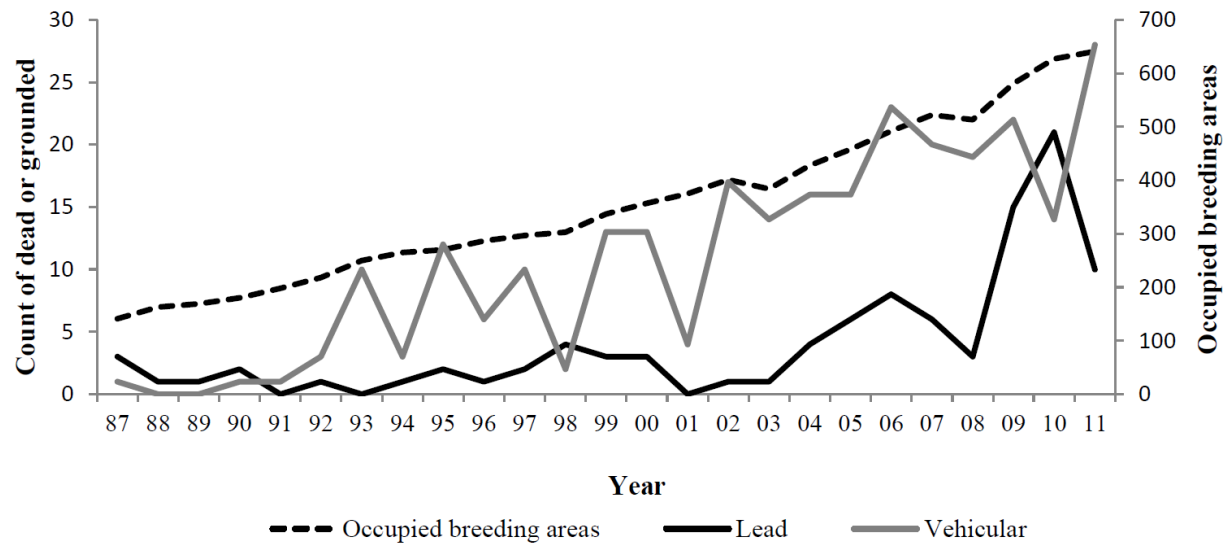


Figure 4.2 Total number of occupied breeding areas and count of dead or grounded bald eagles (*Haliaeetus leucocephalus*) due to vehicular trauma and lead poisoning in Michigan, 1987 to 2011.

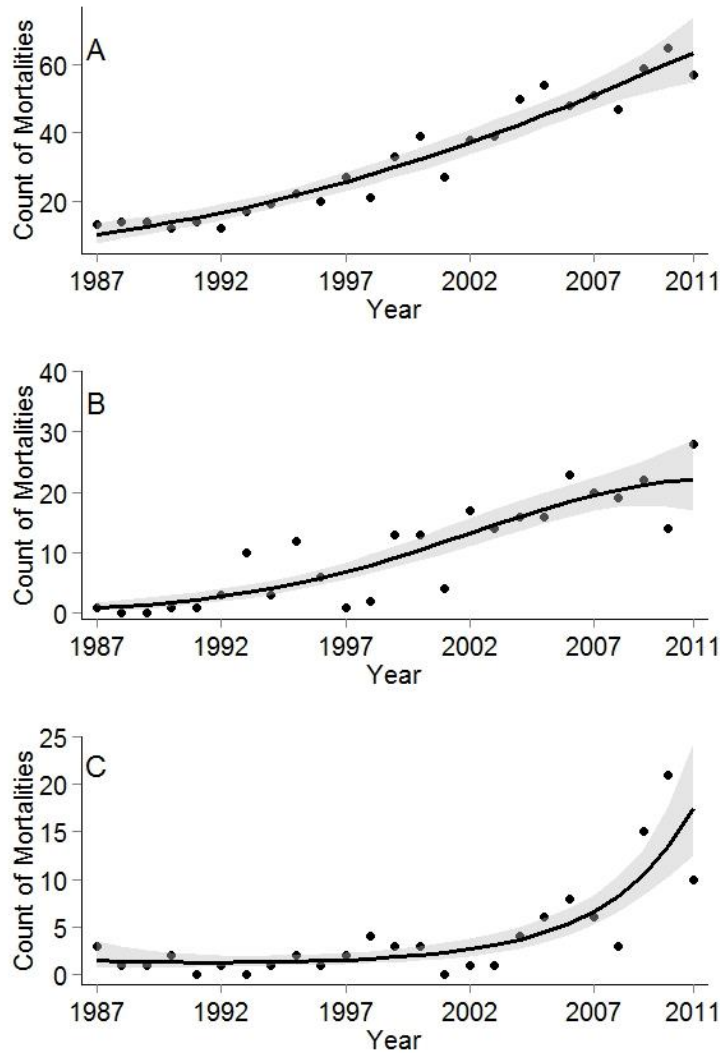


Figure 4.3 Total count of dead or grounded bald eagles (*Haliaeetus leucocephalus*)^A, count of dead or grounded bald eagles due to vehicular trauma^B, and dead or grounded bald eagles due to lead poisoning^C in Michigan, 1987 to 2011. Solid lines represent linear trend models for each mortality classifications ($r^2 = 0.91^A$, 0.78^B , and 0.45^C). Dashed lines represent the regression spline model with breaks at five year intervals for lead poisoning mortalities ($r^2 = 0.74$).

Chapter 5: Summary

The main objective of this study was to use bald eagles (*Haliaeetus leucocephalus*) as environmental indicators of Great Lakes health. Environmental indicators have been proposed as a means to assess ecological integrity, monitoring both chemical and biological stressors. Chemical indicators are used to measure temporal and spatial trends of persistent, bioaccumulative, and toxic substances in biota. In this study, we used concentrations of contaminants in plasma of nestling bald eagles as chemical indicators to quantify direct or indirect tertiary-level contaminant exposure via the food chain. Data for this study were collected through the Michigan Bald Eagle Biomonitoring Project, implemented by the Michigan Department of Environmental Quality as an effort to monitor long-term persistent environmental contaminants and population reproductive output. The use of blood plasma as a sampling medium for environmental contaminants is a minimally invasive technique, which does not harm nestlings. This is especially important for research on a high-profile species such as bald eagles, as well as for a long-term monitoring project with the ability to archive samples for future analyses.

The spatial and temporal trends of polychlorinated biphenyl (PCB) congeners were evaluated in nestling blood plasma collected from 1999–2014. Two hexachlorinated congeners, PCB-138 and 153, were detected with the highest frequency and greatest concentrations throughout all spatial regions of Michigan. This may be due to their higher octanol/ water partition coefficients (7.441–7.751), or increased hydrophobicity, resulting in decreased elimination rates and biomagnification. PCB

138 seems to be more persistent, as no overall trend was detected for any spatial region. PCB 153 however, was shown to be decreasing in all spatial regions. The greater percentage of hexa-chlorinated congeners in remote regions, such as Lake Superior, is likely a result of long-range atmospheric transport. Less-chlorinated congeners such as PCB-52 and 66 however, comprised a greater percentage of total PCB concentrations in nestlings proximate to urbanized areas, such as along the shorelines of Lake Erie. These two congeners also exhibited a clear increase in contribution to total PCB concentrations in nestlings located along Lake Huron, indicating a possible local source. Greater detection frequencies and mean concentrations of individual congeners were observed in nestlings along the shoreline of Lake Erie, despite the least number of samples collected. There were however, either no trends, or decreasing trends detected for all congeners in this spatial region. Toxic equivalents were also greatest in the samples collected from nestlings located on Lake Erie, followed by the other Great Lakes spatial regions.

Future research on PCB congeners in MI bald eagles should determine any patterns in congener composition from West to East in the Upper Peninsula to determine the degree of atmospheric deposition from Western sources, such as China. Lighter-chlorinated congeners will be expected to be closer to the source (i.e. further West), while heavier-chlorinated congeners will be expected to be further East. This analysis may also include samples from Voyageurs National Park, the control site for the Michigan Bald Eagle Biomonitoring Project. In addition, relationships between large-scale environmental variables, such as temperature and snow-fall, could be examined to explain patterns observed in individual congener trend lines.

Analysis of individual PCB congeners highlights the importance of accounting for censored data, or values below the MDL. Removing or simply replacing these data with an arbitrary value may be a valid option when the percentage of non-detected values is very low. Less-frequently detected congeners however, likely contain a greater percentage (20% or greater) of non-detected values. Statistical analysis of these congeners cannot include tests that assume a normal distribution of the variance. Therefore, it is imperative to use non-parametric methods, accounting for censored values, when analyzing and reporting on individual congeners. In addition, the treatment of censored values may grossly bias TEQ results. The removal of censored data may more accurately estimate TEQ concentrations in regions with a small percentage of values below the MDL, but also underestimates TEQ concentrations in regions with a greater percentage of values below the MDL. In contrast, the replacement of censored data with a number, such as $MDL * \frac{1}{2}$, may more accurately estimate TEQ concentrations in regions with greater percentages of values below the MDL, but can over-estimate TEQ concentrations in regions with a lesser percentage of values below the MDL. Choosing a random number between the zero and the MDL is also not an ideal solution, as it drastically underestimated TEQ concentrations in regions with few values below the MDL, but was similar to results using a replacement number ($MDL * \frac{1}{2}$) in regions with greater percentages of values below the MDL. Future reporting on TEQ concentrations using individual PCB congeners should include additional sensitivity analyses to account for these discrepancies. This may require the application of multiple methods depending on the percentage of values below the MDL in a given region.

Archived nestling plasma samples collected between 2000–2012 were used to measure concentrations of the most heavily-used group of flame retardants, brominated diphenyl ethers (BDEs), and three groups of alternative flame retardants, non-BDE Brominated Flame Retardants (NBFRS), Dechloranes, and organophosphate esters (OPs). One tetra-isomer, BDE-47, and two penta-isomers, BDE-99 and 100, contributed the greatest to total BDE concentration. A heavily brominated congener, BDE-209, was also found to be somewhat prevalent in nestling plasma. Concentrations of structurally similar NBFRs found in this study and recent atmospheric studies indicate that they are largely used as replacements to the penta-BDE mixture, in which BDE-47, 99, and 100 were major components, along with the deca-BDE mixture, in which BDE-209 was a major component.

A variety of Dechloranes, or norbornene derivatives of Mirex and Dechlorane Plus, were measured. The major contributing compounds to total Dechlorane concentrations have been found to exhibit a high bioaccumulative potential. Little information is available regarding the production history and current use of these compounds in North America, or globally. This highlights the need for further research to determine the varying degrees of accumulation and metabolism between analogues and transformation products, especially in tertiary-level species. Concentrations of OPs in nestling plasma were two to three orders of magnitude greater than all other groups of flame retardants. The major compounds contributing to total OP concentrations are also those associated with the mixture Firemaster 550, an alternative to the penta-BDE mixture. Certain OPs have been shown to be rapidly metabolize to diester products. Regardless, they have potential to cause adverse

physiological and endocrine effects. Possible sources for flame retardants may be wastewater treatment plants, a fire suppression facility, chemical manufacturing plants, urban runoff, and atmospheric deposition.

Flame retardant studies in the Great Lakes have been limited to the atmosphere, sediments, and a few wildlife species in separate lakes or regions. To better understand degree of persistence and bioaccumulation, future flame retardant studies should include measurements of historic and emerging compounds throughout the food chain, as well as in the atmosphere, sediment, and water. Ideally, these measurements should also be taken within a proximate geographic region, and from all five Great Lakes. Spatial analyses to determine relationships between concentrations and urban centers, WWTPs, and landfills may be informative as well. Further knowledge on the possible toxicological effects of emerging flame retardants is also a major factor required to assess risk posed by these compounds in the Great Lakes for the future.

In addition to chemical indicators, bald eagles have also been proposed as biological indicators of the abundance and distribution of fish-eating and colonial nesting birds. Biological indicators can identify ecological stressors using population matrices that are tied to the fitness of individuals, colonies, and populations of fish-eating birds at multiple geographic scales. This study used mortality as a population vital rate to evaluate major sources of stressors or vulnerability to not only predatory birds, but also all scavenger species throughout Michigan. Anthropogenic-related events were the major cause of mortality. As waterways become unavailable during winter months, eagles become a terrestrial indicator as they switch their prey base,

often feeding on hunter-killed white tailed deer carcasses along roadways. Given this, we found vehicle collisions to be the main source of mortality, especially for females in hunting and snow-melt months. Lead poisoning was the second greatest source of mortality, possibly exacerbated by density-dependent effects due to the growing bald eagle population in Michigan. These results indicate an increasingly direct anthropogenic effect on terrestrial scavenging species. Future research on bald eagle mortality will assess spatial characteristics associated mortality-related events. Life-history factors, such as mid-winter migration, may predispose foraging eagles to mortality-related events along migration routes.

This study effectively utilized bald eagles both as chemical and biological indicators to identify possible stressors to tertiary-level aquatic predators and terrestrial scavenging species in Michigan. The sampling efforts of the Michigan Bald Eagle Biomonitoring Project provide important data on past, current, and future environmental contaminants of concern, further supporting the continued use of bald eagles as sentinels of the Great Lakes ecosystem.

Bibliography

44 IPoCSEHC Mirex. <http://www.inchem.org/documents/ehc/ehc/ehc44.htm>.

Accessed May 2016

Anthony R, Miles K, Ricca M, Estes J (2007) Environmental contaminants in bald eagle eggs from the Aleutian Archipelago. *Environmental Toxicology and Chemistry* preprint:1

Anthony RG, Garrett MG, Schuler CA (1993) Environmental Contaminants in Bald Eagles in the Columbia River Estuary. *JOURNAL OF WILDLIFE MANAGEMENT* 57:10

Bartholomay RC, Davis LC, Fisher JC, Tucker BJ, Raben FA (2012) Water-quality characteristics and trends for selected sites at and near the Idaho National Laboratory, Idaho, 1949-2009. US Geological Survey,

Batterman S, Chernyak S, Gwynn E, Cantonwine D, Jia C, Begnoche L, Hickey JP (2007) Trends of brominated diphenyl ethers in fresh and archived Great Lakes fish (1979–2005). *Chemosphere* 69:444-457

Bellinger DC et al. (2013) Health risks from lead-based ammunition in the environment. *Environmental health perspectives* 121:A178–179

Best DA et al. (2010) Productivity, embryo and eggshell characteristics, and contaminants in bald eagles from the Great Lakes, USA, 1986 to 2000. *Environmental toxicology and chemistry / SETAC* 29:1581-1592

Best DA, Gilbertson M, Hudson H (1990) Proceedings of the Expert Consultation Meeting on Bald Eagles, February 12 and 13, 1990.

- Birnbaum LS, Staskal DF (2004) Brominated flame retardants: cause for concern?
Environmental health perspectives 112:9-17
- Bishop CA, Koster MD, Chek AA, Hussell DJ, Jock K (1995) Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes–St. Lawrence River Basin. Environmental Toxicology and Chemistry 14:491-501
- Bortolotti GR (1984a) Criteria for determining age and sex of nestling bald eagles.
Journal of Field Ornithology 55:467-481
- Bortolotti GR (1984b) Physical development of nestling bald eagles with emphasis on the timing of growth events. The Wilson Bulletin:524-542
- Bosveld ATC, Gradener J, van Den Berg M, Murk AJ, Brouwer A, van Kampen M, Evers EHG (1995) Effects of PCDDs, PCDFs and PCBs in common tern (*Sterna hirundo*) breeding in estuarine and coastal colonies in the Netherlands and Belgium. Environmental Toxicology and Chemistry 14:99-115
- Botts L, Muldoon PR (2005) Evolution of the Great Lakes water quality agreement.
Michigan State University Press East Lansing, Michigan,
- Bowerman W, Best D, Grubb T, Zimmerman G, Giesy J (1998) Trends of Contaminants and Effects in Bald Eagles of the Great Lakes Basin. Environ Monit Assess 53:197-212 doi:10.1023/A:1006068330050
- Bowerman WW, Best DA, Giesy JP, Shieldcastle MC, Meyer MW, Postupalsky S, Sikarskie JG (2003) Associations between regional differences in

- polychlorinated biphenyls and dichlorodiphenyldichloroethylene in blood of nestling bald eagles and reproductive productivity. *Environmental Toxicology and Chemistry* 22:371-376
- Bowerman WW, Giesy JP, Best DA, Kramer VJ (1995) A review of factors affecting productivity of bald eagles in the Great Lakes region: implications for recovery. *Environmental Health Perspectives* 103:51
- Bowerman WW et al. (1994) Observed abnormalities in mandibles of nestling bald eagles *Haliaeetus leucocephalus*. *Bulletin of Environmental Contamination and Toxicology* 53
- Bowerman WW, Roe AS, Gilbertson MJ, Best DA, Sikarskie JG, Mitchell RS, Summer CL (2002) Using bald eagles to indicate the health of the Great Lakes' environment. *Lakes & Reservoirs: Research & Management* 7:183-187
doi:10.1046/j.1440-1770.2002.00186.x
- Brázová Tm, Hanzelová Vr, Miklisová D, Šalgovičová D, Turčeková Ľ (2012) Biomonitoring of polychlorinated biphenyls (PCBs) in heavily polluted aquatic environment in different fish species. *Environmental Monitoring and Assessment : An International Journal Devoted to Progress in the Use of Monitoring Data in Assessing Environmental Risks to Man and the Environment* 184:6553-6561
- Bald Eagle (*Haliaeetus leucocephalus*) (2000) Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North America.
<http://bna.birds.cornell.edu/bna/species/506>. Accessed 10 March 2013

- Buehler SS, Basu I, Hites RA (2004) Causes of variability in pesticide and PCB concentrations in air near the Great Lakes. *Environmental science & technology* 38:414-422
- Burnham KP, Anderson DR (2004) Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods and Research* 33:261–304
doi:10.1177/0049124104268644
- Canada Go, Agency EP (1995) *The Great Lakes: An environmental atlas and resource book*. The Great Lakes National Program Office Chicago, IL, CEPA Environmental Registry SL. <http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=D44ED61E-1>. Accessed 5/20/2016
- ChemSpider (2015) 2,2',5,5'-PCB and 2,3',4,4'-PCB. Royal Society of Chemistry. <http://www.chemspider.com/Chemical-Structure.34189.html?rid=abe7b122-b5da-4e16-af3a-ea4a52b85f35>
<http://www.chemspider.com/Chemical-Structure.33279.html?rid=ff18dd98-0311-4708-8921-fac93007774d>. Accessed 04/22/2016
- Chen D, Hale RC (2010) A global review of polybrominated diphenyl ether flame retardant contamination in birds. *Environment international* 36:800-811
- Chen D, Letcher RJ, Martin P (2012) Flame retardants in eggs of American kestrels and European starlings from southern Lake Ontario region (North America). *Journal of Environmental Monitoring* 14:2870-2876
- Church ME et al. (2006) Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. *Environmental Science and Technology* 40:6143–6150 doi:10.1021/es060765s

- Clarke KR (1999) Nonmetric multivariate analysis in community-level ecotoxicology. *Environmental Toxicology and Chemistry* 18:118-127
- Comba ME, Norstrom RJ, Macdonald CR, Kaiser KL (1993) A Lake Ontario-Gulf of St. Lawrence dynamic mass budget for mirex. *Environmental science & technology* 27:2198-2206
- Commission IJ (2014) Great Lakes Ecosystem Indicator Project Report.
- Committee POPR (2013) Listing of hexabromocyclododecane (C.N.934.2013.TREATIES-XXVII-15).
[http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx#LiveContent\[HBCD\]](http://chm.pops.int/TheConvention/ThePOPs/ListingofPOPs/tabid/2509/Default.aspx#LiveContent[HBCD]).
- Council NR (1978) Kepone/Mirex/Hexachlorocyclopentadiene: an environmental assessment. National Academy of Science, Washington, D.C.
- Covaci A et al. (2006) Hexabromocyclododecanes (HBCDs) in the environment and humans: a review. *Environmental science & technology* 40:3679-3688
- Covaci A, Harrad S, Abdallah MA, Ali N, Law RJ, Herzke D, de Wit CA (2011) Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. *Environment international* 37:532-556
- Custer CM, Custer TW, Dummer PM (2010) Patterns of organic contaminants in eggs of an insectivorous, an omnivorous, and a piscivorous bird nesting on the Hudson River, New York, USA. *Environmental Toxicology and Chemistry* 29:2286-2296

- Donaldson GM, Shutt JL, Hunter P (1999) Organochlorine Contamination in Bald Eagle Eggs and Nestlings from the Canadian Great Lakes. Archives of Environmental Contamination and Toxicology 36:70-80
- Dykstra CR, Meyer MW, Keith Warnke D, Karasov WH, Andersen DE, Bowerman Iv WW, Giesy JP (1998) Low Reproductive Rates of Lake Superior Bald Eagles: Low Food Delivery Rates or Environmental Contaminants? Journal of Great Lakes Research 24:32-44
- Dykstra CR, Meyer MW, Rasmussen PW, Warnke DK (2005) Contaminant concentrations and reproductive rate of Lake Superior bald eagles, 1989–2001. Journal of Great Lakes Research 31:227-235
- Dykstra CR, Meyer MW, Stromborg KL, Keith Warnke D, Bowerman Iv WW, Best DA (2001) Association of Low Reproductive Rates and High Contaminant Levels in Bald Eagles on Green Bay, Lake Michigan. Journal of Great Lakes Research 27:239-251 doi:[http://dx.doi.org/10.1016/S0380-1330\(01\)70637-5](http://dx.doi.org/10.1016/S0380-1330(01)70637-5)
- Dykstra CR, Route WT, Meyer MW, Rasmussen PW (2010) Contaminant Concentrations in Bald Eagles Nesting on Lake Superior, the Upper Mississippi River, and the St. Croix River. Journal of Great Lakes Research 36:561-569
- Eisler R, Belisle AA (1996) Planar PCB hazards to fish, wildlife, and invertebrates: A synoptic review. DTIC Document,
- Elliott et al. (1996a) Biological effects of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. Environmental Toxicology and Chemistry 15:782-793

- Elliott JE (2005) Chlorinated Hydrocarbon Contaminants and Stable Isotope Ratios in Pelagic Seabirds From the North Pacific Ocean. *Archives of Environmental Contamination and Toxicology* 49:89-96
- Elliott JE, Norstrom RJ (1998) Chlorinated hydrocarbon contaminants and productivity of bald eagle populations on the Pacific coast of Canada. *Environmental Toxicology and Chemistry* 17:1142-1153
- Elliott JE et al. (1996b) Biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in bald eagle (*Haliaeetus leucocephalus*) chicks. *Environmental Toxicology and Chemistry* 15:782
- Elliott KH, Cesh LS, Dooley JA, Letcher RJ, Elliott JE (2009) PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. *Science of The Total Environment* 407:3867-3875
doi:<http://dx.doi.org/10.1016/j.scitotenv.2009.02.027>
- DecaBDE phase-out initiative (2009)
<http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/deccadbe.html>.
Accessed May 2016
- <http://www.epa.gov/iur/>. Accessed May 24 2016
- Etter DR, Hollis KM, Van Deelen TR, Ludwig DR, Chelsvig JE, Anchor CL, Warner RE (2002) Survival and movements of white-tailed deer in suburban Chicago, Illinois. *Journal of Wildlife Management* 66:500–510
- Eulaers I et al. (2014) Brominated and phosphorus flame retardants in White-tailed Eagle *Haliaeetus albicilla* nestlings: bioaccumulation and associations with

- dietary proxies ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$). *Science of the Total Environment* 478:48-57
- Fernie K, Bortolotti G, Smits J (2003) Reproductive abnormalities, teratogenicity, and developmental problems in American kestrels (*Falco sparverius*) exposed to polychlorinated biphenyls. *Journal of Toxicology and Environmental Health, Part A* 66:2089-2103
- Fernie KJ, Mayne G, Shutt JL, Pekarik C, Grasman KA, Letcher RJ, Drouillard K (2005a) Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. *Environmental Pollution* 138:485-493
- Fernie KJ et al. (2015) Investigating endocrine and physiological parameters of captive American kestrels exposed by diet to selected organophosphate flame retardants. *Environmental science & technology* 49:7448-7455
- Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Bird DM (2009) Environmentally relevant concentrations of DE-71 and HBCD alter eggshell thickness and reproductive success of American kestrels. *Environmental science & technology* 43:2124-2130
- Fernie KJ, Shutt JL, Letcher RJ, Ritchie IJ, Sullivan K, Bird DM (2008) Changes in reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to environmentally relevant levels of the polybrominated diphenyl ether mixture, DE-71. *Toxicological sciences* 102:171-178
- Fernie KJ, Shutt JL, Mayne G, Hoffman D, Letcher RJ, Drouillard KG, Ritchie IJ (2005b) Exposure to polybrominated diphenyl ethers (PBDEs): changes in

- thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). *Toxicological Sciences* 88:375-383
- Ferrer M, Newton IAN, Casado EVA (2006) How to test different density-dependent fecundity hypotheses in an increasing or stable population. *Journal of Animal Ecology* 75:111–117
- Fielder DG, Schaeffer JS, Thomas MV (2007) Environmental and Ecological Conditions Surrounding the Production of Large Year Classes of Walleye (*Sander vitreus*) in Saginaw Bay, Lake Huron. *Journal of Great Lakes Research* 33:118-132
- Fisher IJ, Pain DJ, Thomas VG (2006) A review of lead poisoning from ammunition sources in terrestrial birds. *Biological Conservation* 131:421–432
- Franson JC, Russell RE (2014) Lead and eagles: demographic and pathological characteristics of poisoning, and exposure levels associated with other causes of mortality. *Ecotoxicology* 23:1722–1731
- Fredricks TB et al. (2011) Effects on tree swallows exposed to dioxin-like compounds associated with the Tittabawassee River and floodplain near Midland, Michigan, USA. *Environmental Toxicology and Chemistry* 30:1354-1365
- Freedman P, Monson B (1989) Great Lakes Water Quality Agreement. *Water Environment and Technology* 1
- Friend M, Franson JC (1999) Field manual of wildlife diseases. General field procedures and diseases of birds. U.S. Geological Survey, Biological Resources Division, National Wildlife Health Center, Madison, WI, USA

- Friend M, Franson JC, Anderson WL Biological and societal dimensions of lead poisoning in birds in the USA. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) Ingestion of lead from spent ammunition: implications for wildlife and humans, Boise, ID, USA, 2009. The Peregrine Fund, pp 34–60
- Gandhi N et al. (2015) Is mirex still a contaminant of concern for the North American Great Lakes? *Journal of Great Lakes Research* 41:1114-1122
- Garrett MG, Watson JW, Anthony RG (1993) Bald eagle home range and habitat use in the Columbia River estuary. *The Journal of wildlife management*:19-27
- Gauthier LT, Hebert CE, Weseloh DC, Letcher RJ (2007) Current-use flame retardants in the eggs of herring gulls (*Larus argentatus*) from the Laurentian Great Lakes. *Environmental science & technology* 41:4561-4567
- Gauthier LT, Hebert CE, Weseloh DC, Letcher RJ (2008a) Dramatic changes in the temporal trends of polybrominated diphenyl ethers (PBDEs) in herring gull eggs from the Laurentian Great Lakes: 1982–2006. *Environmental science & technology* 42:1524-1530
- Gauthier LT, Potter D, Hebert CE, Letcher RJ (2008b) Temporal trends and spatial distribution of non-polybrominated diphenyl ether flame retardants in the eggs of colonial populations of Great Lakes herring gulls. *Environmental science & technology* 43:312-317
- Gewurtz SB, Backus SM, Bhavsar SP, McGoldrick DJ, de Solla SR, Murphy EW (2011) Contaminant biomonitoring programs in the Great Lakes region: Review of approaches and critical factors. *Environmental Reviews* 19:162-184

- Gilbertson M (1992) Proceedings of the Third Expert Consultation Meeting on Bald Eagles in the Great Lakes Basin, February 25 to 26, 1992, International Joint Commission, Windsor, Ontario.
- Gilbertson M, Kubiak T, Ludwig J, Fox G (1991) Great Lakes embryo mortality, edema, and deformities syndrome (GLEMEDS) in colonial fish-eating birds: similarity to chick-edema disease. *Journal of toxicology and environmental health* 33:455-520
- Gill CE, Elliott JE (2003) Influence of Food Supply and Chlorinated Hydrocarbon Contaminants on Breeding Success of Bald Eagles. *Ecotoxicology* 12:95-111
- Gioia R, Dachs J, Nizzetto L, Lohmann R, Jones KC (2013) Atmospheric Transport, Cycling and Dynamics of Polychlorinated Biphenyls (PCBs) from Source Regions to Remote Oceanic Areas. *ACS SYMPOSIUM SERIES*:3-18
- Golden NH, Rattner BA (2003) Ranking Terrestrial Vertebrate Species for Utility in Biomonitoring and Vulnerability to Environmental Contaminants. *Reviews of Environmental Contamination and Toxicology*:67-136
- Gregor DJ, Gummer WD (1989) Evidence of atmospheric transport and deposition of organochlorine pesticides and polychlorinated biphenyls in Canadian Arctic snow. *Environmental Science & Technology* 23:561-565
doi:10.1021/es00063a008
- Grier JW (1982) Ban of DDT and subsequent recovery of reproduction in bald eagles. *Science* 218:1232-1235

- Grimalt JO, Van Drooge BL, Ribes A, Vilanova RM, Fernandez P, Appleby P (2004) Persistent organochlorine compounds in soils and sediments of European high altitude mountain lakes. *Chemosphere* 54:1549-1561
- Grubb TG, Lopez RG (2000) Food habits of bald eagles wintering in northern Arizona. *Journal of Raptor Research* 34:287–292
- Hahm J, Manchester-Neesvig JB, DeBord D, Sonzogni WC (2009) Polybrominated diphenyl ethers (PBDEs) in Lake Michigan forage fish. *Journal of Great Lakes Research* 35:154-158
- Haig SM et al. (2014) The persistent problem of lead poisoning in birds from ammunition and fishing tackle. *The Condor* 116:408–428
- Harris MC, Sleeman JM (2007) Morbidity and mortality of bald eagles (*Haliaeetus leucocephalus*) and peregrine falcons (*Falco peregrinus*) admitted to the Wildlife Center of Virginia, 1993-2003. *Journal of Zoo and Wildlife Medicine* 38:62-66
- Hartman KJ, Margraf FJ (1992) Effects of prey and predator abundances on prey consumption and growth of walleyes in western Lake Erie. *Transactions of the American Fisheries Society* 121:245-260
- Haukås M, Hylland K, Nygård T, Berge JA, Mariussen E (2010) Diastereomer-specific bioaccumulation of hexabromocyclododecane (HBCD) in a coastal food web, Western Norway. *Science of the total environment* 408:5910-5916
- He JX et al. (2015) Coupling age-structured stock assessment and fish bioenergetics models: a system of time-varying models for quantifying piscivory patterns

- during the rapid trophic shift in the main basin of Lake Huron. *Canadian Journal of Fisheries and Aquatic Sciences* 72:7-23
- He JX et al. (2012) Lake Trout Status in the Main Basin of Lake Huron, 1973–2010. *North American Journal of Fisheries Management* 32:402-412
- Health USDo, Services H (2000) Toxicological profile for polychlorinated biphenyls (PCBs). Atlanta, GA: Agency for Toxic Substances and Disease Registry
- Hebert CE, Norstrom RJ, Weseloh DC (2000) A quarter century of environmental surveillance: the Canadian Wildlife Service's Great Lakes herring gull monitoring program. *Environmental Reviews* 7:147-166
- Hebert CE, Norstrom RJ, Zhu J, Macdonald CR (1999) Historical Changes in PCB Patterns in Lake Ontario and Green Bay, Lake Michigan, 1971 to 1982, from Herring Gull Egg Monitoring Data. *Journal of Great Lakes Research* 25:220-233
- Helander B, Bignert A, Asplund L (2008) Using Raptors as Environmental Sentinels: Monitoring the White-tailed Sea Eagle *Haliaeetus albicilla* in Sweden. *Ambio* 37:425-431
- Helsel DR (2011) Statistics for censored environmental data using Minitab and R vol 77. John Wiley & Sons,
- Henny CJ, Kaiser JL, Grove RA, Johnson BL, Letcher RJ (2009) Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. *Ecotoxicology* 18:802-813

- Hernández LM, Fernández MA, González MJ (1989) Total PCBs and PCB congeners in spanish imperial eagle eggs. *Bulletin of Environmental Contamination and Toxicology* 43:725-732
- Hickey JP, Batterman SA, Chernyak SM (2006) Trends of chlorinated organic contaminants in Great Lakes trout and walleye from 1970 to 1998. *Archives of Environmental Contamination and Toxicology* 50:97-110
- Hites RA (2004) Polybrominated diphenyl ethers in the environment and in people: a meta-analysis of concentrations. *Environmental science & technology* 38:945-956
- Hoff RM, Muir DCG, Grift NP (1992) Annual cycle of polychlorinated biphenyls and organohalogen pesticides in air in southern Ontario. 2. Atmospheric transport and sources. *Environmental Science & Technology* 26:276-283
- Hoffman DJ (1995) *Handbook of ecotoxicology*. Lewis Publishers, Boca Raton :
- Hoffman DJ, Rattner BA, Sileo L, Docherty D, Kubiak TJ (1987) Embryotoxicity, teratogenicity, and aryl hydrocarbon hydroxylase activity in Forster's terns on Green Bay, Lake Michigan. *Environmental research* 42:176-184
- Holden A et al. (2009) Unusual hepta-and octabrominated diphenyl ethers and nonabrominated diphenyl ether profile in California, USA, peregrine falcons (*Falco peregrinus*): More evidence for brominated diphenyl ether-209 debromination. *Environmental Toxicology and Chemistry* 28:1906-1911
- Houde M, Berryman D, de Lafontaine Y, Verreault J (2014) Novel brominated flame retardants and dechloranes in three fish species from the St. Lawrence River, Canada. *Science of the Total Environment* 479:48-56

- Hsu Y-K, Holsen TM, Hopke PK (2003) Locating and quantifying PCB sources in Chicago: receptor modeling and field sampling. *Environmental science & technology* 37:681-690
- Hunt WG, Burnham W, Parish CN, Burnham KK, Mutch B, Oaks JL (2006) Bullet Fragments in deer remains: implications for lead exposure in avian scavengers. *Wildlife Society Bulletin* 34:167–170
- Hunt WG et al. (2009) Lead bullet fragments in venison from rifle-killed deer: potential for human dietary exposure. *PloS one* 4:e5330
- Iwata H, Tanabe S, Ueda K, Tatsukawa R (1995) Persistent organochlorine residues in air, water, sediments, and soils from the Lake Baikal region, Russia. *ENVIRONMENTAL SCIENCE AND TECHNOLOGY -WASHINGTON DC-* 29:792
- Johansson AK, Sellström U, Lindberg P, Bignert A, De Wit CA (2009) Polybrominated diphenyl ether congener patterns, hexabromocyclododecane, and brominated biphenyl 153 in eggs of peregrine falcons (*Falco peregrinus*) breeding in Sweden. *Environmental Toxicology and Chemistry* 28:9-17
- Johnson-Restrepo B, Kannan K (2009) An assessment of sources and pathways of human exposure to polybrominated diphenyl ethers in the United States. *Chemosphere* 76:542-548
- Johnson CK, Kelly TR, Rideout BA (2013) Lead in ammunition: a persistent threat to health and conservation. *EcoHealth* 10:455–464
- Jones KC, De Voogt P (1999) Persistent organic pollutants (POPs): state of the science. *Environmental Pollution* 100:209-221

- Kelly TR et al. (2014) Causes of mortality and unintentional poisoning in predatory and scavenging birds in California. Veterinary record open 1:e000028
- Kendall RJ et al. (1996) An ecological risk assessment of lead shot exposure in non-waterfowl avian species: upland game birds and raptors. Environmental Toxicology and Chemistry 15:4–20
- Kierkegaard A, Björklund J, Fridén U (2004) Identification of the flame retardant decabromodiphenyl ethane in the environment. Environmental Science & Technology 38:3247-3253
- Kim EY, Goto R, Iwata H, Masuda Y, Tanabe S, Fujita S (1999) Preliminary survey of lead poisoning of Steller's sea eagle (*Haliaeetus pelagicus*) and white-tailed sea eagle (*Haliaeetus albicilla*) in Hokkaido, Japan. Environmental Toxicology and Chemistry 18:448–451
- Kitchell JF et al. (2000) Sustainability of the Lake Superior fish community: interactions in a food web context. Ecosystems 3:545-560
- Koistinen J, Koivusaari J, Nuuja I, Vuorinen PJ, Paasivirta J, Giesy JP (1997) 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin equivalents in extracts of Baltic white-tailed sea eagles. Environmental toxicology and chemistry 16:1533-1544
- Krueger B, Krueger K (2003) US Fish and Wildlife Service fact sheet: secondary pentobarbital poisoning in wildlife.
<http://cpharm.vetmed.vt.edu/USFWS/USFWSFPentobarbFactSheet.pdf>.
Accessed 03 March 2013
- Krüger O, Lindström J (2001) Habitat heterogeneity affects population growth in goshawk *Accipiter gentilis*. Journal of animal Ecology 70:173–181

- Lafrancois BM, Riley SC, Blehert DS, Ballmann AE (2011) Links between type E botulism outbreaks, lake levels, and surface water temperatures in Lake Michigan, 1963–2008. *Journal of Great Lakes Research* 37:86–91
- Lam JC, Lau RK, Murphy MB, Lam PK (2009) Temporal trends of hexabromocyclododecanes (HBCDs) and polybrominated diphenyl ethers (PBDEs) and detection of two novel flame retardants in marine mammals from Hong Kong, South China. *Environmental science & technology* 43:6944-6949
- Lamon III EC, Carpenter SR, Stow CA (2000) Depuration of PCBs in the Lake Michigan Ecosystem. *Ecosystems* 3:332-343
- Langelier K (1993) Barbiturate poisoning in twenty-nine bald eagles. In: Redig PT (ed) *Raptor Biomedicine*. University of Minnesota Press, Minneapolis, MN, USA, pp 231–232
- Lee L (2013) NADA: Nondetects And Data Analysis for environmental data. <https://CRAN.R-project.org/package=NADA>
- Leith KF, Bowerman WW, Wierda MR, Best DA, Grubb TG, Sikarske JG (2010) A comparison of techniques for assessing central tendency in left-censored data using PCB and p,p'DDE contaminant concentrations from Michigan's Bald Eagle Biosentinel Program. *Chemosphere* 80:7-12
- Letcher R, Chen D, Chu S, Gauthier L (2011) Organophosphate triester flame retardant and phosphoric acid diester metabolite analysis and spatial and temporal trends in herring gulls from the North American Great Lakes. *Organohalogen Compd* 73:1257-1260

- Lewin WC, Arlinghaus R, Mehner T (2006) Documented and potential biological impacts of recreational fishing: insights for management and conservation. *Reviews in Fisheries Science* 14:305–367
- Liu H-H, Hu Y-J, Luo P, Bao L-J, Qiu J-W, Leung KM, Zeng EY (2014) Occurrence of halogenated flame retardants in sediment off an urbanized coastal zone: association with urbanization and industrialization. *Environmental science & technology* 48:8465-8473
- Liu L-Y, Venier M, Salamova A, Hites RA (2016) A Novel Flame Retardant in the Great Lakes Atmosphere: 3, 3', 5, 5'-Tetrabromobisphenol A Bis (2, 3-dibromopropyl) Ether. *Environmental Science & Technology Letters*
- Ludwig JP et al. (1996) Deformities, PCBs, and TCDD-equivalents in Double-crested Cormorants (*Phalacrocorax auritus*) and Caspian Terns (*Hydroprogne caspia*) of the upper Great Lakes 1986–1991: Testing a cause-effect hypothesis. *Journal of Great Lakes Research* 22:172-197
- Lundstedt-Enkel K, Lek PM, Lundstedt T, Örberg J (2006) QSBMR-Quantitative structure biomagnification relationships: physicochemical and structural descriptors important for the biomagnification of organochlorines and brominated flame retardants. *Journal of chemometrics* 20:392-401
- Ma Y, Salamova A, Venier M, Hites RA (2013) Has the phase-out of PBDEs affected their atmospheric levels? Trends of PBDEs and their replacements in the Great Lakes atmosphere. *Environmental science & technology* 47:11457-11464

- Ma Y, Venier M, Hites RA (2011) 2-Ethylhexyl tetrabromobenzoate and bis (2-ethylhexyl) tetrabromophthalate flame retardants in the Great Lakes atmosphere. *Environmental science & technology* 46:204-208
- Martinson SC, Drouillard KG, Verreault J (2016) Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird. *Environmental Research* 146:73-84
- McKernan MA, Rattner BA, Hale RC, Ottinger MA (2009) Toxicity of polybrominated diphenyl ethers (de-71) in chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), and American kestrel (*Falco sparverius*) embryos and hatchlings. *Environmental Toxicology and Chemistry* 28:1007-1017
- McKinney MA, Cesh LS, Elliott JE, Williams TD, Garcelon DK, Letcher RJ (2006) Brominated flame retardants and halogenated phenolic compounds in North American west coast bald eaglet (*Haliaeetus leucocephalus*) plasma. *Environmental science & technology* 40:6275-6281
- Meijer SN, Ockenden WA, Sweetman A, Breivik K, Grimalt JO, Jones KC (2003) Global Distribution and Budget of PCBs and HCB in Background Surface Soils: Implications for Sources and Environmental Processes. *Environmental Science & Technology* 37:667-672
- Meijers A, Van Der Leer RC (1976) The occurrence of organic micropollutants in the river Rhine and the river Maas in 1974. *Water Research* 10:597-604
- Melymuk L et al. (2014) From the city to the lake: Loadings of PCBs, PBDEs, PAHs and PCMs from Toronto to Lake Ontario. *Environmental science & technology* 48:3732-3741

Michigan Traffic Crash Facts (2011)

http://publications.michigantrafficcrashfacts.org/2011/2011MTCF_vol1.pdf.

Accessed 10 March 2013

Möller A, Xie Z, Caba A, Sturm R, Ebinghaus R (2011) Organophosphorus flame retardants and plasticizers in the atmosphere of the North Sea. *Environmental Pollution* 159:3660-3665

Murphy C, Bhavsar S, Gandhi N (2012) Contaminants in Great Lakes Fish: historic, current, and emerging concerns. *Great Lakes Fisheries Policy and Management: A Binational Perspective* 2

Nadjafzadeh M, Hofer H, Krone O (2013) The link between feeding ecology and lead poisoning in white-tailed eagles. *Journal of Wildlife Management* 77:48–57

Nagayama J et al. (1998) Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. *Chemosphere* 37:1789-1793

Neumann K Bald eagle lead poisoning in winter. In: Watson RT, Fuller M, Pokras M, Hunt WG (eds) *Ingestion of lead from spent ammunition: implications for wildlife and humans*, Boise, ID, USA, 2009. The Peregrine Fund, pp 210–218

Newton I (1979) *Population ecology of raptors*. Buteo Books, Vermillion, SD, USA

Nyberg E, Danielsson S, Eriksson U, Faxneld S, Miller A, Bignert A (2014) Spatio-temporal trends of PCBs in the Swedish freshwater environment 1981-2012. *Ambio* 43:45-57 doi:<http://dx.doi.org/10.1007/s13280-014-0561-4>

- Offenberg JH, Baker JE (1997) Polychlorinated biphenyls in Chicago precipitation: enhanced wet deposition to near-shore Lake Michigan. *Environmental science & technology* 31:1534-1538
- Pagel JE, Sharpe PB, Garcelon DK, Little AE, Taylor SK, Faulkner KR, Gorbics CS (2012) Exposure of bald eagles to lead on the northern Channel Islands, California. *Journal of Raptor Research* 46:168–176
- Pattee OH, Bloom PH, Scott JM, Smith MR (1990) Lead hazards within the range of the California Condor. *Condor* 92:931–937
- Pattee OH, Wiemeyer SN, Mulhern BM, Sileo L, Carpenter JW (1981) Experimental lead-shot poisoning in bald eagles. *Journal of Wildlife Management* 45:806–810
- Pekarik C, Weseloh DV (1999) Organochlorine contaminants in herring gull eggs from the Great Lakes, 1974-1995: Change point regression analysis and short-term regression. *Journal of Planning Literature* 14
- Peverly AA, Salamova A, Hites RA (2014) Air is still contaminated 40 years after the Michigan chemical plant disaster in St. Louis, Michigan. *Environmental science & technology* 48:11154-11160
- Pirard C, De Pauw E (2007) Absorption, disposition and excretion of polybrominated diphenyl ethers (PBDEs) in chicken. *Chemosphere* 66:320-325
- Pittman HT, Bowerman WW, Grim LH, Grubb TG, Bridges WC, Wierda MR (2015) Using nestling plasma to assess long-term spatial and temporal concentrations of organochlorine compounds in bald eagles within Voyageurs National Park, Minnesota, USA. *Chemosphere* 123:79-86

Postupalsky S Raptor reproductive success: Some problems with methods, criteria, and terminology. In: Hamerstrom FN, Harrell BE, ROhlendorff R (eds) Management of Raptors, Fort Collins, CO, USA, March 22–24 1974. Raptor Report 2. Raptor Conservation Technologies, pp 21–31

Postupalsky S (1985) The bald eagles' return. *Journal of Natural History* 87:62–63

Qiu X, Marvin CH, Hites RA (2007) Dechlorane plus and other flame retardants in a sediment core from Lake Ontario. *Environmental science & technology* 41:6014-6019

Quortrup ER, Sudheimer RL (1943) Detection of botulinus toxin in the bloodstream of wild ducks. *Journal of the American Veterinary Medical Association* 102:264–266

R Development Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Rattner BA, Hatfield JS, Melancon MJ, Custer TW, Tillitt DE (1994) Relation among cytochrome p450, ah-active PCB congeners and dioxin equivalents in pipping black-crowned night-heron embryos. *Environmental Toxicology and Chemistry* 13:1805-1812

Ross PS, Couillard CM, Ikonomou MG, Johannessen SC, Lebeuf M, Macdonald RW, Tomy GT (2009) Large and growing environmental reservoirs of Deca-BDE present an emerging health risk for fish and marine mammals. *Marine Pollution Bulletin* 58:7-10

Route WT, Dykstra CR, Rasmussen PW, Key RL, Meyer MW, Mathew J (2014) Patterns and trends in brominated flame retardants in bald eagle nestlings

- from the Upper Midwestern United States. *Environmental science & technology* 48:12516-12524
- Russell RE, Franson JC (2014) Causes of mortality in eagles submitted to the National Wildlife Health Center 1975–2013. *Wildlife Society Bulletin* 38:697–704
- Safe S (1990) Polychlorinated Biphenyls (PCBs), Dibenzo-p-Dioxins (PCDDs), Dibenzofurans (PCDFs), and Related Compounds: Environmental and Mechanistic Considerations Which Support the Development of Toxic Equivalency Factors (TEFs). *CRC Critical Reviews in Toxicology* 21:51-88
- Salamova A, Hites RA (2010) Evaluation of tree bark as a passive atmospheric sampler for flame retardants, PCBs, and organochlorine pesticides. *Environmental science & technology* 44:6196-6201
- Salamova A, Hites RA (2011) Discontinued and alternative brominated flame retardants in the atmosphere and precipitation from the Great Lakes basin. *Environmental science & technology* 45:8698-8706
- Salamova A, Hites RA (2012) Brominated and chlorinated flame retardants in tree bark from around the globe. *Environmental science & technology* 47:349-354
- Salamova A, Ma Y, Venier M, Hites RA (2014) High levels of organophosphate flame retardants in the Great Lakes atmosphere. *Environmental Science & Technology Letters* 1:8-14
- Sanderson JT, Elliott JE, Norstrom RJ, Whitehead PE, Hart LE, Cheng KM, Bellward GD (1994) Monitoring biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in great blue heron chicks

- (*Ardea herodias*) in British Columbia. *Journal of Toxicology and Environmental Health, Part A Current Issues* 41:435-450
- Scheringer M (2009) Long-range transport of organic chemicals in the environment. *Environmental Toxicology and Chemistry* 28:677-690
- Scheuhammer AM, Money SL, Kirk DA, Donaldson G (2003) Lead fishing sinkers and jigs in Canada: review of their use patterns and toxic impacts on wildlife vol 108. Canadian Wildlife Service Occasional Paper 108, Ottawa, Canada.
- Scheuhammer AM, Templeton DM (1998) Use of stable isotope ratios to distinguish sources of lead exposure in wild birds. *Ecotoxicology* 7:37-42
- Schlechtriem C, Fliedner A, Schäfers C (2012) Determination of lipid content in fish samples from bioaccumulation studies: contributions to the revision of guideline OECD 305. *Environmental Sciences Europe* 24:13
- Selzer M, Bureau W (2008) The Michigan Department of Environmental Quality Biennial Remedial Action Plan Update for the Saginaw River/Bay Area of Concern.
- Senthil Kumar K, Kannan K, Giesy JP, Masunaga S (2002) Distribution and elimination of polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls, and p, p'-DDE in tissues of bald eagles from the upper peninsula of Michigan. *Environmental science & technology* 36:2789-2796
- Service USFaW (2015) Saginaw River and Bay Natural Resource Damage Assessment, Dredging Project.
- <http://www.fws.gov/midwest/es/ec/NRDA/SaginawNRDA/dredge.html>.
- Accessed 04/21/2016 2016

- Seston RM et al. (2010) Tissue-based risk assessment of great blue heron (*Ardea herodias*) exposed to PCDD/DF in the Tittabawassee River floodplain, Michigan, USA. *Environmental Toxicology and Chemistry* 29:2544-2558
- Seston RM et al. (2012) Dietary- and tissue-based exposure of belted kingfisher to PCDFs and PCDDs in the Tittabawassee River floodplain, Midland, MI, USA. *Environmental Toxicology and Chemistry* 31:1158-1168
- Sheldon LS, Hites RA (1978) Organic compounds in the Delaware River. *Environmental Science & Technology* 12:1188-1194
- Shen L et al. (2014) Identification and occurrence of analogues of dechlorane 604 in Lake Ontario sediment and their accumulation in fish. *Environmental science & technology* 48:11170-11177
- Shen L et al. (2011a) Historic trends of dechloranes 602, 603, 604, dechlorane plus and other norbornene derivatives and their bioaccumulation potential in Lake Ontario. *Environmental science & technology* 45:3333-3340
- Shen L et al. (2011b) Dechloranes 602, 603, 604, Dechlorane Plus, and Chlordene Plus, a newly detected analogue, in tributary sediments of the Laurentian Great Lakes. *Environmental science & technology* 45:693-699
- Shen L et al. (2010) Identification and screening analysis of halogenated norbornene flame retardants in the Laurentian Great Lakes: Dechloranes 602, 603, and 604. *Environmental science & technology* 44:760-766
- Simon KL (2013) Bald Eagle (*Haliaeetus leucocephalus*) population productivity and density dependent effects in Michigan, 1961-2010. Thesis, University of Maryland

- Sprunt A, W.B. Robertson Jr, Postupalsky A, Hensel RJ, Knoder CE, Ligas FJ (1973) Comparative productivity of six bald eagle populations. Transactions of the North American Wildlife and Natural Resources Conference 38:96–106
- Stauber E, Finch N, Talcott PA, Gay JM (2010) Lead poisoning of bald (*Haliaeetus leucocephalus*) and golden (*Aquila chrysaetos*) eagles in the US inland Pacific Northwest region- An 18-year retrospective study: 1991-2008. Journal of Avian Medicine and Surgery 24:279–287
- Stow CA, Carpenter SR, Eby LA, Amrhein JF, Hesselberg RJ (1995) Evidence That PCBs Are Approaching Stable Concentrations In Lake Michigan Fishes. Ecological Applications 5:248-260
- Su G, Greaves AK, Gauthier L, Letcher RJ (2014a) Liquid chromatography-electrospray–tandem mass spectrometry method for determination of organophosphate diesters in biotic samples including Great Lakes herring gull plasma. Journal of Chromatography A 1374:85-92
- Su G, Letcher RJ, Moore JN, Williams LL, Martin PA, de Solla SR, Bowerman WW (2015) Spatial and temporal comparisons of legacy and emerging flame retardants in herring gull eggs from colonies spanning the Laurentian Great Lakes of Canada and United States. Environmental research 142:720-730
- Su H, Wu F, Zhang R, Zhao X, Mu Y, Feng C, Giesy JP (2014b) Toxicity reference values for protecting aquatic birds in China from the effects of polychlorinated biphenyls. In: Reviews of Environmental Contamination and Toxicology volume. Springer, pp 59-82

- Sudharsan K, Riley SJ, Winterstein SR (2006) Relationship of autumn hunting season to the frequency of deer-vehicle collisions in Michigan. *Journal of Wildlife Management* 70:1161–1164
- Sühling R, Barber JL, Wolschke H, Kötke D, Ebinghaus R (2015) Fingerprint analysis of brominated flame retardants and Dechloranes in North Sea sediments. *Environmental research* 140:569-578
- Sun P, Basu I, Blanchard P, Brice KA, Hites RA (2007) Temporal and spatial trends of atmospheric polychlorinated biphenyl concentrations near the Great Lakes. *Environmental science & technology* 41:1131-1136
- Sun P, Basu I, Hites RA (2006) Temporal trends of polychlorinated biphenyls in precipitation and air at Chicago. *Environmental science & technology* 40:1178-1183
- Sun Y-x et al. (2012) Hexabromocyclododecane in terrestrial passerine birds from e-waste, urban and rural locations in the Pearl River Delta, South China: Levels, biomagnification, diastereoisomer-and enantiomer-specific accumulation. *Environmental pollution* 171:191-198
- Sundberg SE, Ellington JJ, Evans JJ (2006) A simple and fast extraction method for organochlorine pesticides and polychlorinated biphenyls in small volumes of avian serum. *Journal of chromatography B, Analytical technologies in the biomedical and life sciences* 831:99-104
- Sverko E et al. (2009) Compounds structurally related to Dechlorane Plus in sediment and biota from Lake Ontario (Canada). *Environmental science & technology* 44:574-579

- Sverko E et al. (2011) Dechlorane Plus and related compounds in the environment: a review. *Environmental science & technology* 45:5088-5098
- Symes CT, Kruger TL (2012) The persistence of an apex avian predator, Verreaux's eagle, *Aquila verreauxii*, in a rapidly urbanizing environment. *South African Journal of Wildlife Research* 42:45–53
- Tachikawa R, Wakimoto T, Okada K Water pollution by organic phosphate ester plasticizers and flame retardants. In: *JSWPR Symposium Series*, 1975. vol 9. pp 7-12
- Tasdemir Y, Vardar N, Odabasi M, Holsen TM (2004) Concentrations and gas/particle partitioning of PCBs in Chicago. *Environmental Pollution* 131:35-44
- Thomas VG (2013) Lead-free hunting rifle ammunition: product availability, price, effectiveness, and role in global wildlife conservation. *Ambio* 42:737–745
- Tillitt DE et al. (1992) Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the great lakes. *Environmental Toxicology and Chemistry* 11:1281-1288
- Tomy GT et al. (2004) Biomagnification of α - and γ -hexabromocyclododecane isomers in a Lake Ontario food web. *Environmental science & technology* 38:2298-2303
- Tomy GT et al. (2013) Dechlorane plus monoadducts in a lake ontario (Canada) food web and biotransformation by lake trout (*Salvelinus namaycush*) liver microsomes. *Environmental Toxicology and Chemistry* 32:1376-1381

- Trinogga A, Fritsch G, Hofer H, Krone O (2013) Are lead-free hunting rifle bullets as effective at killing wildlife as conventional lead bullets? A comparison based on wound size and morphology. *Science of the Total Environment* 443:226–232
- U.S. Fish and Wildlife Service (1983) Northern States Bald Eagle Recovery Plan. Denver, Colorado, USA
- U.S. Fish and Wildlife Service (1986) Use of lead shot for hunting migratory birds in the United States. US Department of the Interior, Fish and Wildlife Service, Washington, D.C
- U.S. Fish and Wildlife Service (2007) Endangered and threatened wildlife and plants; final rule to remove the bald eagle in the lower 48 states from the list of endangered and threatened wildlife. *Federal Register* 72:37346–37372
- UNEP (2009) Report of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants.
- van den Berg M et al. (1998) Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. *Environmental health perspectives* 106:775–792
- van den Berg M et al. (2006) The 2005 World Health Organization reevaluation of human and Mammalian toxic equivalency factors for dioxins and dioxin-like compounds. *Toxicological sciences : an official journal of the Society of Toxicology* 93:223–241
- van den Berg M et al. (1994) Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-*P*-dioxins (PCDDs) and dibenzofurans

- (PCDFs) in the cormorant (*Phalacrocorax carbo*) after in ovo exposure. *Environmental Toxicology and Chemistry* 13:803-816
- Van den Steen E, Covaci A, Jaspers V, Dauwe T, Voorspoels S, Eens M, Pinxten R (2007) Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (*Sturnus vulgaris*). *Environmental Pollution* 148:648-653
- van der Veen I, de Boer J (2012) Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* 88:1119-1153
- Vander Zanden MJ, Rasmussen JB (1996) A Trophic Position Model of Pelagic Food Webs: Impact on Contaminant Bioaccumulation in Lake Trout. *Ecological Monographs* 66:451-477 doi:10.2307/2963490
- Velleux M, Burns S, DePinto JV, Hassett JP (1995) A screening-level mass balance analysis of mirex transport and fate in the Oswego River. *Journal of Great Lakes Research* 21:95-111
- Venier M, Hites RA (2008) Flame retardants in the atmosphere near the Great Lakes. *Environmental science & technology* 42:4745-4751
- Venier M, Ma Y, Hites RA (2012) Bromobenzene flame retardants in the Great Lakes atmosphere. *Environmental science & technology* 46:8653-8660
- Venier M, Salamova A, Hites RA (2015) Halogenated Flame Retardants in the Great Lakes Environment. *Accounts of Chemical Research* 48:1853-1861 doi:10.1021/acs.accounts.5b00180
- Venier M, Wierda M, Bowerman WW, Hites RA (2010) Flame retardants and organochlorine pollutants in bald eagle plasma from the Great Lakes region.

- Chemosphere 80:1234-1240
- doi:<http://dx.doi.org/10.1016/j.chemosphere.2010.05.043>
- Verboven N, Verreault J, Letcher RJ, Gabrielsen GW, Evans NP (2009) Differential investment in eggs by Arctic-breeding glaucous gulls (*Larus hyperboreus*) exposed to persistent organic pollutants. *The Auk* 126:123-133
- Verreault J, Letcher RJ, Muir DCG, Chu S, Gebbink WA, Gabrielsen GW (2005) New organochlorine contaminants and metabolites in plasma and eggs of glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic. *Environmental Toxicology and Chemistry* 24:2486-2499
- Wang P et al. (2016) Sources and environmental behaviors of Dechlorane Plus and related compounds—A review. *Environment international* 88:206-220
- Warner SE, Britton EE, Becker DN, Coffey MJ (2014) Bald eagle lead exposure in the Upper Midwest. *Journal of Fish and Wildlife Management* 5:208–216
- Watson JW (2002) Comparative home ranges and food habits of bald eagles nesting in four aquatic habitats in western Washington. *Northwestern Naturalist*:101-108
- Whelan GE, Johnson JE (2005) Successes and Failures of Large-Scale Ecosystem Manipulation Using Hatchery Production: The Upper Great Lakes Experience. *American Fisheries Society Symposium* 3-32
- Wickham H (2009) *ggplot2: Elegant Graphics for Data Analysis*. Springer, Verlag, New York
- Wiemeyer SN, Bunck CM, Stafford CJ (1993) Environmental contaminants in bald eagle eggs—1980–84—and further interpretations of relationships to

- productivity and shell thickness. Archives of Environmental Contamination and Toxicology 24:213-227 doi:10.1007/bf01141351
- Wierda MR, Leith KF, Grubb TG, Sikarskie JG, Best DA, Bowerman W (2016) Using bald eagles to track spatial (1999–2008) and temporal (1987–1992, 1999–2003, and 2004–2008) trends of contaminants in Michigan's aquatic ecosystems. Environmental Toxicology and Chemistry
- Wood PB, Buehler DA, Byrd MA Raptor status report–bald eagle. In: Proceedings of the Southeast Raptor Management Symposium and Workshop, Washington, DC, 1990. National Wildlife Federation, pp 13–21
- Yang R, Wei H, Guo J, Li A (2012) Emerging brominated flame retardants in the sediment of the Great Lakes. Environmental science & technology 46:3119-3126
- Yang R, Wei H, Guo J, McLeod C, Li A, Sturchio NC (2011) Historically and currently used Dechloranes in the sediments of the Great Lakes. Environmental science & technology 45:5156-5163
- Yule AM, Barker IK, Austin JW, Moccia RD (2006) Toxicity of *Clostridium botulinum* type E neurotoxin to Great Lakes fish: implications for avian botulism. Journal of Wildlife Diseases 42:479–493
- Zhou SN et al. (2010) Liquid chromatography–atmospheric pressure photoionization tandem mass spectrometry for analysis of 36 halogenated flame retardants in fish. Journal of Chromatography A 1217:633-641
- Zhu LY, Hites RA (2005) Brominated flame retardants in sediment cores from Lakes Michigan and Erie. Environmental science & technology 39:3488-3494

