

MICHIGAN WILDLIFE CONTAMINANT
TREND MONITORING

**Nestling Bald Eagle Mercury Report:
Spatial Trends
1999-2012
Temporal Trends
1986-2012**

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Abstract:

The bald eagle (*Haliaeetus leucocephalus*) is a widely distributed bird of prey throughout Michigan. It has been extensively studied and is susceptible to the effects of environmental contaminants. As a long-lived apex predator, the species is exposed to the effects of biomagnification, and analysis of tissue samples can produce valuable information about organisms positioned lower in the food chain. In addition, bald eagles are territorial nesters; therefore, nestlings provide a representation of the contaminant levels of the local environment. Mercury (Hg) can have negative effects on the environment, and feathers of exposed bald eagles have been analyzed to monitor Hg levels. Nestling bald eagle feather samples were collected throughout the state of Michigan from 1986 to 2012 and analyzed for concentrations of elemental Hg. Data were used to evaluate spatial and temporal trends of Hg throughout the state of Michigan. Results show that Hg decreased from 1986 to 2008 with a slight increase between 2009 and 2012. Overall, remediation has positively affected the Great Lakes region. However, with changing climate, land-use practices, and human population trends, management strategies should be planned cautiously, particularly in Michigan's Upper Peninsula and Lake Superior shoreline.

INTRODUCTION

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

The survival of the species became a topic of concern in the 1960s after a dramatic decrease in the population due to a combination of birds being shot and trapped as varmints, and the exposure and effects of anthropogenic pollutants (dichlorodiphenyltrichloroethane [DDT] and polychlorinated biphenyls [PCB]). Eagle numbers plummeted drastically with only 52 breeding pairs recorded in the state of Michigan in 1961. Bald eagles were placed on the federal Endangered Species List as Endangered in 1976 throughout its range with the exception of Alaska. This mandated protection afforded the eagle reprieve from shooting, and once DDT and PCB were officially outlawed in 1972 and 1976, respectively, the population began to rebound. Nationwide monitoring efforts were put into place to evaluate population growth with aerial and ground surveys. As of 2012, eagle populations are estimated to be greater than 650 active breeding pairs in Michigan and numbers are still improving. The total number of young produced each year has also increased from 34 in 1961 to 721 in 2012 (Figure 1). Productivity for each breeding pair was determined by the proportion of total number of young (young) to the number of occupied breeding areas (occupied) each year (Postupalsky, 1974). A productivity value equal to 0.7 young/occupied indicates a stable population and the federal recovery goal associated with a healthy population is 1.0 young/occupied (Sprunt et al., 1973).

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

Elemental Hg and its compounds have no known metabolic function, but have shown a measurable increase in animal tissues in aquatic food chains. Hg typically enters regions of open water by direct deposition or transportation through runoff (Hurley et al., 1995; Landis and Keeler, 2002; Rudd, 1995). More specifically, Hg is found in the environment by the following anthropogenic and natural mechanisms: natural deposits in the soil, anthropogenic point sources, and atmospheric deposition (Chan et al., 2003; Driscoll et al., 2007). Once in anaerobic regions, such as those commonly found in wetlands and lake sediments, Hg can be converted to methylmercury (MeHg). MeHg is a highly toxic compound that readily accumulates in organisms and biomagnifies up the food chain to concentrations that exceed those measured in surface water (Chasar et al., 2009; Driscoll et al., 2007; Evers et al., 2011; Rolfus et al., 2011; Scheuhammer, 1987; Wiener et al., 2003). MeHg is a documented mutagen, teratogen, and carcinogen, and causes embryocidal, cytochemical, and histopathological effects (Eisler, 2007). As this organic compound biomagnifies, the highest MeHg levels are found in tertiary predators

such as the bald eagle. This contamination has given cause for concern for the health of humans and piscivorous wildlife.

MeHg contamination of fish, and the associated impairments to water usage, can diminish the recreational, economic, and nutritional benefits of freshwater resources. Eighty percent of fish advisories across the nation are attributed to Hg (United States Environmental Protection Agency [USEPA], 2009). Sediment cores from lakes in both hemispheres show that the net Hg deposition has increased threefold since preindustrial times due to an increase in anthropogenic inputs (Bindler et al., 2001; Lamborg et al., 2002; Lindberg et al., 2007). Studies have indicated that Hg methylation has increased by the availability of organic carbon that is stored in organic matter, and actively broken down by microbial activity (Winfrey and Rudd, 1990). This cycle is especially prevalent in flooded reservoirs and other dynamic wetland habitats.

Wetlands are the primary sites of Hg methylation, which expose biota to elevated levels of contamination (Branfireun et al., 2005; Brigham et al., 2009; Chasar et al., 2009; Hurley et al., 1995; Wiener et al., 2006). Michigan contains roughly 15 percent wetland habitat. The level of MeHg in fish has an inverse relationship with pH and alkalinity (Wiener et al., 1990). The Great Lakes are naturally lower in alkalinity, which reduces the area's buffering capacity. According to current climate models, if atmospheric CO₂ continues to trend upward, the lake pH may decline, resulting in conditions that enhance the efficiency of MeHg uptake by fish (Rodgers and Beamish, 1983). Compared to inorganic Hg, MeHg is less volatile and is more bioavailable for uptake. In addition, Hg methylation has a direct relationship with temperature (Wright and Hamilton, 1982). According to a study conducted by Myers et al. (2009), both average annual minimum and maximum temperatures increased in Michigan over a 37-year period.

Signs of Hg poisoning in birds include muscular incoordination, falling, slowness, fluffed feathers, calmness, withdrawal, hyporeactivity, hypoactivity, and eyelid drooping. Changes in behavior may disrupt and negatively affect foraging and nesting behavior (Jagoe et al., 2002). In wild birds, environmental MeHg exposure may be associated with a higher potential for infection and decreased growth (Harris et al., 2010; Scheuhammer et al., 2007). Hg concentrations in eggs have also been implicated in impaired hatchability and embryonic mortality in a number of bird species (Scheuhammer et al., 2007; Wiener et al., 2003). In the case of bald eagles, MeHg readily enters the blood stream after ingesting contaminated prey. The kidneys, liver, spleen, muscle, and brain are targeted resulting in Hg toxicosis. Eventually the Hg is either stored in feathers or eliminated (Fournier et al., 2002).

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

In 1999, the Michigan Department of Environmental Quality (MDEQ) implemented the Michigan Bald Eagle Biomonitoring Project under the Clean Michigan Initiative. In addition to population productivity and individual bird biometrics, this long-term monitoring effort provides information about persistent environmental contaminants including PCBs, organochlorine pesticides such as DDT, and heavy metals such as Hg. Blood and feather samples and biometrics are taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of relevant measures. Long-term monitoring has allowed for the determination

that bald eagle productivity is increasing spatially and temporally in congruence with the decline of PCB and DDT values below lowest observed adverse effect levels. However, emergent issues have become a concern such as Hg bioavailability, the related contamination in the aquatic ecosystem, and whether its presence negatively impacts human and bald eagle populations.

The primary objective of this study was to measure Hg in nestling bald eagle feathers for the purpose of determining spatial and temporal trends throughout the state of Michigan. Temporal trends were evaluated from 1986 to 2012, and the last 15 years of data were compared at four spatial scales. Spatial analyses were conducted at four spatial scales for 1999-2003, 2004-2008 and 2009-2012. Lastly, data were used to isolate areas of concern. This report corrects analytical errors made in the previous report (Wierda et al., 2009) and provides the results of more recent analysis.

METHODS

Study Area

The state has been divided into sampling units with a sampling goal of 20 percent of Michigan's watersheds each year (Figure 2). With this design, the entire state was sampled every five years. Feather samples were taken from nestling bald eagles throughout the state on an annual basis to evaluate spatial and temporal trends of Hg.

Spatial and Temporal Analysis

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

The Statewide spatial scale compared Inland (IN) and Great Lakes (GL) breeding areas. Great Lakes breeding areas were those areas within 8 kilometers (km) (approximately 5 miles) of Great Lakes shorelines, and along tributaries open to Great Lakes fish. Inland breeding areas were areas located beyond 8 km from shorelines and not along Great Lakes tributaries (Bowerman et al., 1994; Roe, 2001; Bowerman et al., 2003).

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

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

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

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

Field Methods

Aerial Surveys

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

Nestling Eagle Capture

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

Sample Collection

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

Lab Methods

Feather Preparation

Feather samples were washed in a sealed plastic bag using approximately five percent diluted Citranox® and tap water. Next, feathers were rinsed three times with tap water, and three times with reverse-osmosis water. Feathers were then placed into a 2 milliliter (mL) cryogenic vial,

covered with folded Chemwipes® that were secured with rubber bands, and stored at -30°C for 24 hours. Once chilled, the feathers were lyophilized for 72 hours after which they were placed in a vacuum or stored with desiccant until digestion. Prior to analysis, 0.05 grams ($\pm 0.005\text{g}$) of each sample were weighed out and placed into 100 mL glass test tubes. Feathers were digested with 10 mL of trace metal grade nitric acid and each tube was capped using a glass marble. Tubes were placed in a block heater at 80°C for 30 minutes. The samples were then removed from heat and allowed to reach room temperature for 30 minutes. Digested feathers were placed into 250 mL glass jars, diluted to 1:20 (acid to water) using deionized water, sealed with Parafilm®, capped, and stored at room temperature until analysis.

Hg Analysis

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

Hg concentrations were quantified and verified for quality assurance/quality control using a Hg Reference Standard Solution by Fisher Scientific and prediction curves. An initial standard stock solution of 1,000 milligrams per kilogram (mg/kg) (± 1 mg/kg) was used to make five serial dilution standards of 1, 2, 5, 10, and 20 mg/kg. A standard curve was generated from the standards, and quality checks were performed after every five samples to assure correct instrument standard readings. Optimal recovery rates were set within 85 to 115 percent of the original Hg standard curve.

Statistical Methods

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

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

RESULTS

Spatial Trends

1999-2003

A total of 416 feather samples were collected from nestling bald eagles throughout the state of Michigan between 1999 and 2003, and were analyzed for Hg. These samples were representative of 410 individual breeding areas. Comparisons of Hg concentrations in nestling feathers were made at the Statewide, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales.

Statewide

Hg concentrations in feather samples taken from Great Lakes breeding areas were similar to those taken from Inland sites ($F = 1.09^{1, 414}$, $p = 0.2973$). Geometric means for Hg concentrations were 3.17 and 3.32 mg/kg for GL and IN, respectively (Table 1).

Subpopulation

Hg concentrations did not vary significantly among feathers from nestling eagles at the Subpopulation spatial scale ($F = 1.58^{5, 410}$, $p = 0.1637$). Geometric mean values were ranked in the following order from lowest to highest: LE (1.04 mg/kg), IN-UP (2.54 mg/kg), LH (2.72 mg/kg), LS (3.60 mg/kg), LM (3.93 mg/kg), and IN-LP (4.43 mg/kg) (Table 1).

Great Lakes Watershed

Hg concentrations in samples at the Great Lakes watersheds spatial scale were similar ($F = 0.96^{9, 406}$, $p = 0.4746$). Geometric mean concentrations of Hg were ranked in the following order from lowest to highest: GL-LE (1.04 mg/kg), IN-UP-LM (2.24 mg/kg), GL-LH (2.72 mg/kg), IN-UP-LS (3.11 mg/kg), GL-LS (3.60 mg/kg), GL-LM (3.93 mg/kg), IN-LP-LM (4.30 mg/kg), and IN-LP-LH (4.49 mg/kg) (Table 1).

Individual Watersheds

Hg concentrations did not vary significantly among Individual Watersheds ($F = 1.41^{43, 372}$, $p = 0.0507$). Hg concentrations for Individual Watersheds ranged from 0.23 to 11.64 mg/kg. HUCs were grouped by their affiliation with Great Lakes areas (GL-HUC), Inland areas (IN-HUC), or both (M-HUC). Hg concentrations did not vary among Grouped HUCs ($F = 0.70^{2, 413}$, $p = 0.4970$). The Mixed HUCs (M-HUC) had the highest geometric mean (3.85 mg/kg) followed by IN-HUC and GL-HUC with values of 2.66 and 2.17 mg/kg, respectively.

2004-2008

A total of 384 feather samples were collected from nestling bald eagles throughout the state of Michigan between 2004 and 2008, and were analyzed for Hg. These samples were representative of 223 individual breeding areas. Comparisons of Hg concentrations in nestling feathers were made at the Statewide, Subpopulation, Great Lakes Watershed, and Individual Watershed spatial scales.

Statewide

Hg concentrations in feather samples taken from Great Lakes breeding areas were significantly lower than those taken from Inland sites ($F = 29.20^{1, 382}$, $p > 0.0001$). Geometric means for Hg concentrations were 0.59 and 1.05 mg/kg for GL and IN, respectively (Table 2).

Subpopulation

Hg concentrations varied significantly among feathers from nestling eagles at the Subpopulation spatial scale ($F = 6.92^{5, 378}$, $p < 0.0001$). Post-hoc analysis showed statistically similar Hg values in samples from all Inland (IN-LP, IN-UP) sites, and breeding areas along Lake Erie (LE). Consequently, these spatial scales had the highest geometric mean values of 1.21, 1.18, and 0.96 mg/kg for IN-UP, LE, and IN-LP, respectively. Great Lakes sites along Lakes Huron (LH), Michigan (LM), and Superior (LS) had significantly different concentrations than IN-UP. In the Lower Peninsula, Hg concentrations along LH and LM were significantly different than IN-LP. When comparing all of the Great Lakes areas, LE, LH, LM, and LS had no significant differences ($LSD = 1.97$, $df = 378$, $p \leq 0.05$). Geometric mean Hg concentrations for LH, LM, and LS were 0.66 mg/kg, 0.60 mg/kg, and 0.49 mg/kg, respectively (Table 2).

Great Lakes Watershed

Hg concentrations in samples at the Great Lakes watersheds spatial scale varied significantly ($F = 4.49^{8, 375}$, $p < 0.0001$). Inland areas associated with Lake Huron did not differ between peninsulas (IN-UP-LH, IN-LP-LH). However, Hg concentrations were significantly different between IN-LP-LH and Lake Huron sites within 8 km of the shoreline (GL-LH). IN-UP-LM and IN-LP-LM did not vary significantly, but both areas were different from GL-LM ($LSD = 1.97$, $df = 375$, $p \leq 0.05$). Geometric mean concentrations of Hg were ranked in the following order from lowest to highest: GL-LS (0.49 mg/kg), GL-LM (0.60 mg/kg), GL-LH (0.66 mg/kg), IN-UP-LM (0.87 mg/kg), IN-LP-LH (0.88 mg/kg), IN-LP-LM (1.11 mg/kg), GL-LE (1.18 mg/kg), IN-UP-LH (1.79 mg/kg), IN-UP-LS (1.87 mg/kg) (Table 2).

Individual Watersheds

Hg concentrations did not vary significantly among Individual Watersheds ($F = 1.06^{44, 339}$, $p = 0.37$). Hg concentrations for Individual Watersheds ranged from 0.01 to 4.64 mg/kg. HUCs were grouped by their affiliation with Great Lakes areas (GL-HUC), Inland areas (IN-HUC), or both (M-HUC). Hg concentrations did not vary among Grouped HUCs ($F = 2.93^{2, 381}$, $p > 0.05$). Great Lakes HUCs (GL-HUC) had the highest geometric mean (0.86 mg/kg) followed by IN-HUC and M-HUC with values of 0.81 and 0.77 mg/kg, respectively.

2009-2012

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

Statewide

Hg concentrations in feather samples taken from Great Lakes breeding areas were not significantly different from those taken from Inland sites ($F = 3.87^{1, 224}$, $p = 0.0505$). Geometric means for Hg concentrations were 1.41 and 1.62 mg/kg for GL and IN, respectively (Table 3).

Subpopulation

Hg concentrations varied significantly among feathers from nestling eagles at the Subpopulation spatial scale ($F = 5.21^{5, 220}$, $p < 0.0002$). Post-hoc analysis showed statistically similar Hg values in samples from all Inland (IN-LP, IN-UP) sites, and breeding areas along Lakes Superior (LS) and Huron (LH). These spatial scales had the highest geometric mean values of 2.90, 1.82, 1.72, and 1.40 mg/kg for LS, IN-UP, IN-LP, and LH respectively. Great Lakes sites along Lake Michigan (LM) (1.27 mg/kg) had significantly lower concentrations than IN-UP (1.82 mg/kg), but were similar to LH (1.4 mg/kg). In the Lower Peninsula, Hg concentrations along LM and LE (0.16 mg/kg) were statistically similar and lower than IN-LP ($LSD = 1.97$, $df = 220$, $p \leq 0.05$) (Table 3).

Great Lakes Watershed

Hg concentrations in samples at the Great Lakes watersheds spatial scale varied significantly ($F = 2.65^{10, 215}$, $p = 0.0045$). Inland areas associated with Lake Huron did not differ between peninsulas (IN-UP-LH, IN-LP-LH), and were significantly similar to Lake Huron sites within 8 km of the shoreline (GL-LH). IN-UP-LM and IN-LP-LM did not vary significantly. However, GL-LM sites were significantly lower than IN-UP-LM sites ($LSD = 1.97$, $df = 215$, $p \leq 0.05$). Geometric mean concentrations of Hg were ranked in the following order from lowest to highest: IN-LP-LE (0.04 mg/kg), GL-LE (0.20 mg/kg), GL-LM (1.27 mg/kg), GL-LH (1.40 mg/kg), IN-LP-LM (1.41 mg/kg), IN-UP-LM (1.57 mg/kg), IN-LP-LH (1.93 mg/kg), IN-UP-LS (2.19 mg/kg), and GL-LS (2.90 mg/kg) (Table 3).

Individual Watersheds

Hg concentrations varied significantly among 47 Individual Watersheds ($F = 1.61^{46, 178}$, $p = 0.0151$). Further post-hoc analysis did not show any significant differences. Geometric mean Hg concentrations for Individual Watersheds ranged from 0.04 to 4.97 mg/kg ($LSD = 5.71$, $df = 178$, $p \leq 0.05$). HUCs were grouped by their affiliation with Great Lakes areas (GL-HUC), Inland areas (IN-HUC), or both (M-HUC). Hg concentrations varied among Grouped HUCs ($F = 3.21^{2, 222}$, $p = 0.0424$). Concentrations of Hg in Great Lakes HUCs (GL-HUC) were significantly lower than IN-HUC with geometric means of 2.23 and 2.3 mg/kg, respectively. The geometric mean concentration of Hg in the Mixed HUCs (1.76 mg/kg) was not significantly different than the concentrations in the GL-HUC and IN-HUC.

Temporal Trends

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

Analysis of Temporal Changes

At the end of the 2012 sampling cycle, the Michigan Bald Eagle Biosentinel Project completed three consecutive five-year cycles (T2, T3, and T4). To assess the utility of continued

monitoring, additional post-hoc analyses were conducted to isolate areas of concern as well as areas where remediation and responsible stewardship has proven beneficial to the aquatic ecosystem. Due to the nature of the Project's sampling regime, not all results were significant because of small sample sizes; however, important temporal trends and differences were observed within spatial scales. The Great Lakes Watershed spatial scale was used for analyses because changes in Hg appeared to be localized among individual breeding areas. Statewide and Subpopulation trends can still be viewed in Figure 3.

T2 vs. T3

There were statistically significant decreases in Hg for all Great Lakes Watershed spatial scales ($p < 0.0001$) with the exception of GL-LE and IN-UP-LH, which had observed decreases that were not significant, which was likely due to small sample sizes ($p \geq 0.05$). The average decrease in Hg concentration was 0.82 mg/kg between T2 and T3. Fifty-four of 800 breeding areas that were analyzed had an observable increase in Hg concentrations between T2 and T3. Two of 12 Great Lakes sites along Lake Erie (GL-LE) had an increase in Hg (0.62 and 2.43 mg/kg). Four of 133 GL-LH sites had an increase in Hg (range = 0.30 to 16.74 mg/kg). GL-LM had 12 of 120 breeding areas with increasing Hg concentrations (0.24 to 18.37 mg/kg). Eight of 115 GL-LS sites had increasing Hg (0.17 to 6.20 mg/kg). Eight of 147 Lower Peninsula Inland sites associated with Lake Huron (IN-LP-LH) were increasing (0.25 to 2.66 mg/kg). None of the three IN-UP-LH sites that were analyzed had an increase in Hg concentration. Five of 79 and 12 of 127 Inland sites were increasing in IN-LP-LM and IN-UP-LM, respectively (0.06 to 2.15 mg/kg and 0.04 to 3.93 mg/kg). Lastly, two of 57 IN-UP-LS sites analyzed for Hg were increasing over time (0.89 and 1.98 mg/kg) (Figure 4).

T3 vs. T4

There were observable increases in all Great Lake Watershed spatial scales with the exception of GL-LE, which had a slight decrease in Hg. There were statistically significant increases in Hg for the following Great Lakes Watershed spatial scales ($p < 0.05$) GL-LS, GL-LH, and IN-LP-LH. Increases were also observed in GL-LM, IN-LP-LM, IN-UP-LM, IN-UP-LH, and IN-UP-LS that were not significant ($p \geq 0.05$). The average increase in Hg concentration was 0.18 mg/kg between T3 and T4. Eighty-seven of 329 breeding areas that were analyzed had an observable increase in Hg concentrations between T3 and T4. Two of 5 Great Lakes sites along Lake Erie (GL-LE) had an increase in Hg (0.03 and 1.06 mg/kg). Thirteen of 45 GL-LH sites had an increase in Hg (0.02 to 2.71 mg/kg). GL-LM had 14 of 44 breeding areas with increasing Hg concentrations (0.02 to 2.19 mg/kg). Twelve of 45 GL-LS sites had increasing Hg (0.02 to 1.43 mg/kg). Eight of 44 Lower Peninsula Inland sites associated with Lake Huron (IN-LP-LH) were increasing (0.06 to 1.14 mg/kg). There was only 1 site in IN-UP-LH, so no comparison was available. Five of 29 and 9 of 39 Inland sites were increasing in IN-LP-LM and IN-UP-LM, respectively (0.02 to 2.15 mg/kg and 0.62 to 7.31 mg/kg). Lastly, 7 of 23 IN-UP-LS sites analyzed for Hg were increasing over time (0.28 to 6.36 mg/kg) (Figure 4).

Additional Analyses

Additional post-hoc analyses were conducted to observe which specific breeding areas had the five highest Hg concentrations for T2, T3, and T4.

All five breeding areas representing the highest Hg concentrations during T2 were located in the Upper Peninsula. Three of 5 breeding areas were located along the Great Lakes shoreline in Marquette and Baraga Counties. The nest in Marquette County was sampled at least twice during T2, and had two of the highest values of Hg (25.57 and 41.86 mg/kg). The

Baraga County nest had a Hg concentration of 33.19 mg/kg. The additional two breeding areas were associated with Lake Michigan and located in Mackinaw (GL-LM) and Menominee (IN-UP-LM) Counties (33.06 and 40.29 mg/kg, respectively).

Four of 5 breeding areas with the highest Hg concentrations during T3 were located in the Upper Peninsula and associated with Lake Superior. These areas were located in Alger (two sites: GL-LS and IN-UP-LS), Marquette (GL-LS), and Baraga (GL-LS) Counties with Hg concentrations of 10.56, 12.03, 12.60, and 11.03 mg/kg, respectively. The fourth area had a Hg concentration of 10.15 mg/kg and was located in Missaukee County (IN-LP-LM). At least one area in Alger, Baraga, and Mackinaw Counties had increasing Hg between T2 and T3. Although the Hg concentrations in the breeding area in Marquette County was not increasing over the entire time period, it was increasing within T3, and it had one of the highest observed Hg concentrations three times during the T2 and T3 time periods.

All five breeding areas representing the highest Hg concentrations during T4 were located in the Upper Peninsula. Four of five breeding areas were located along the Great Lakes shoreline in Marquette, Baraga, and Chippewa Counties. The nests in Marquette and Baraga Counties were sampled at least twice between T3 and T4, and had two of the highest values of Hg in each time period. The Marquette nest had values of 12.60 and 9.52 mg/kg for T3 and T4, respectively. The Baraga County nest had Hg concentrations of 11.03 and 20.97 mg/kg, respectively. The fifth nest was associated with IN-UP-LS in Gogebic County (17.35 mg/kg).

DISCUSSION

This study reports spatial trends in Hg concentrations found in nestling bald eagle feathers during the Michigan Bald Eagle Biomonitoring Project's 2009-2012 sampling cycle, and temporal trends between 1986-1993, 1999-2003, 2004-2008, and 2009-2012. Because Hg biomagnifies, values associated with organisms at high trophic levels provide valuable information about the aquatic ecosystem. Theoretical Hg concentrations can be derived from examining Biomagnification Factors that are defined by the known ratio of a contaminant concentration in biota to that in the surrounding water when the biota was exposed to contaminated food (Nowell et al., 2010).

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

Utility of Bald Eagles as Sentinels

The utility of the bald eagle as a sentinel species remains apparent with the quantity and quality of spatial and temporal trend data that align with other intensive studies. Because bald eagles are known to demethylate Hg, these data may provide a conservative estimate of actual Hg effects to eagles (Scheuhammer et al., 2007). As evidenced by the steady increase in the Michigan bald eagle's productivity and expanding population, Hg is currently not negatively affecting the birds themselves. However, some individual breeding areas may be of concern due to localized increases in Hg concentrations, and/or having consistently high concentrations that

may translate into high Hg in nearby fisheries. Future studies should focus on localized increased contamination, in addition to the statewide data.

Recommendations

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

Based on the results stated in this report, we recommend the following:

- Continue monitoring bald eagle productivity and the collection of nestling feather samples statewide to assess trends and effects of Hg levels as the environment continues to change.
- Increase sampling efforts in Michigan’s Upper Peninsula in Great Lakes shoreline and Inland breeding areas to identify and monitor areas of high Hg concentrations.

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Table 1. Sample size, geometric mean, and range concentration of Hg in nestling bald eagle feathers collected in Michigan during the 1999-2003 time period.

		Sample Size ^a	Geometric Mean	Range ^b (mg/kg)
Statewide				
Great Lakes	GL	190	3.17	ND-41.86
Inland	IN	226	3.32	ND-40.29
Subpopulation				
Inland Upper Peninsula	IN-UP	116	2.54	ND-40.29
Lake Superior	LS	59	3.60	ND-41.86
Lake Michigan	LM	54	3.93	0.47-33.06
Lake Erie	LE	7	1.04	ND-13.53
Lake Huron	LH	70	2.72	ND-20.92
Inland Lower Peninsula	IN-LP	110	4.43	0.04-19.84
Great Lakes Watershed				
Inland Upper Peninsula Lake Superior	IN-UP-LS	30	3.11	0.19-18.12
Inland Upper Peninsula Lake Michigan	IN-UP-LM	80	2.24	ND-40.29
Great Lakes Lake Superior	GL-LS	59	3.60	ND-41.86
Inland Upper Peninsula Lake Huron	IN-UP-LH	x	x	x
Inland Lower Peninsula Lake Huron	IN-LP-LH	75	4.49	0.04-17.42
Great Lakes Lake Michigan	GL-LM	54	3.93	0.47-33.06
Great Lakes Lake Erie	GL-LE	7	1.04	ND-13.53
Inland Lower Peninsula Lake Erie	IN-LP-LE	x	x	x
Great Lakes Lake Huron	GL-LH	70	2.72	ND-20.92
Inland Lower Peninsula Lake Michigan	IN-LP-LM	35	4.30	0.29-19.84

a. Values denoted with an "x" have a sample size of ≤ 1 .

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

Table 2. Sample size, geometric mean, and range concentration of Hg in nestling bald eagle feathers collected in Michigan during the 2004-2008 time period. Spatial scales with an “*” had Hg concentrations that varied significantly.

		Sample Size ^a	Geometric Mean	Range ^b (mg/kg)
Statewide*				
Great Lakes	GL	190	0.59	ND-12.60
Inland	IN	194	1.05	ND-12.03
Subpopulation*				
Inland Upper Peninsula	IN-UP	78	1.21	ND-12.03
Lake Superior	LS	56	0.49	ND-12.60
Lake Michigan	LM	66	0.60	ND-4.08
Lake Erie	LE	5	1.18	0.75-1.99
Lake Huron	LH	63	0.66	ND-4.08
Inland Lower Peninsula	IN-LP	116	0.96	ND-10.15
Great Lakes Watershed*				
Inland Upper Peninsula Lake Superior	IN-UP-LS	27	1.87	ND-12.03
Inland Upper Peninsula Lake Michigan	IN-UP-LM	47	0.87	ND-8.38
Great Lakes Lake Superior	GL-LS	56	0.49	ND-12.60
Inland Upper Peninsula Lake Huron	IN-UP-LH	2	1.79	1.23-2.61
Inland Lower Peninsula Lake Huron	IN-LP-LH	72	0.88	ND-6.03
Great Lakes Lake Michigan	GL-LM	66	0.60	ND-4.08
Great Lakes Lake Erie	GL-LE	5	1.18	0.75-1.99
Inland Lower Peninsula Lake Erie	IN-LP-LE	x	x	x
Great Lakes Lake Huron	GL-LH	63	0.66	ND-4.08
Inland Lower Peninsula Lake Michigan	IN-LP-LM	44	1.11	ND-10.15

a. Values denoted with an “x” have a sample size of ≤ 1 .

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

Table 3. Sample size, geometric mean, and range concentration of Hg in nestling bald eagle feathers collected in Michigan during the 2009-2012 time period. Spatial scales with an “*” had Hg concentrations that varied significantly.

		Sample Size ^a	Geometric Mean	Range ^b (mg/kg)
Statewide				
Great Lakes	GL	138	1.41	ND-20.97
Inland	IN	88	1.62	ND-17.35
Subpopulation*				
Lake Superior	LS	42	2.90	0.17-20.97
Inland Upper Peninsula	IN-UP	43	1.82	ND-17.35
Inland Lower Peninsula	IN-LP	43	1.72	ND-9.20
Lake Huron	LH	41	1.40	ND-8.55
Lake Michigan	LM	42	1.27	ND-7.73
Lake Erie	LE	15	0.16	ND-2.92
Great Lakes Watershed*				
Inland Upper Peninsula Lake Superior	IN-UP-LS	17	2.19	0.24-17.35
Inland Upper Peninsula Lake Michigan	IN-UP-LM	24	1.57	ND-8.70
Great Lakes Lake Superior	GL-LS	42	2.90	0.17-20.97
Inland Upper Peninsula Lake Huron	IN-UP-LH	x	x	x
Inland Lower Peninsula Lake Huron	IN-LP-LH	27	1.93	ND-9.20
Great Lakes Lake Michigan	GL-LM	42	1.27	ND-7.73
Great Lakes Lake Erie	GL-LE	13	0.20	ND-2.67
Inland Lower Peninsula Lake Erie	IN-LP-LE	2	0.04	ND-2.92
Great Lakes Lake Huron	GL-LH	41	1.40	ND-8.55
Inland Lower Peninsula Lake Michigan	IN-LP-LM	16	1.41	ND-5.35

a. Values denoted with a “x” have a sample size of ≤ 1 .

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

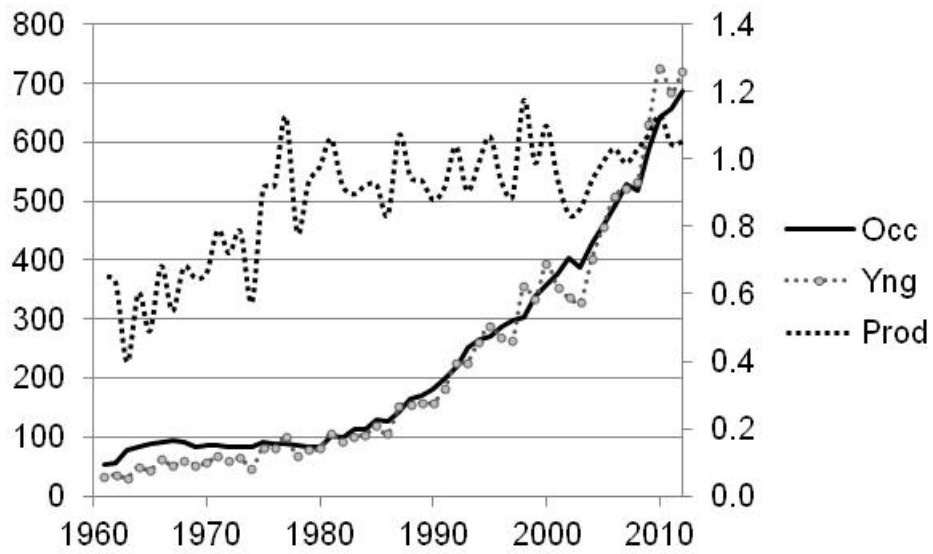


Figure 1. Relationship of bald eagle occupied nests, young produced, and productivity per year from 1961 to 2012.

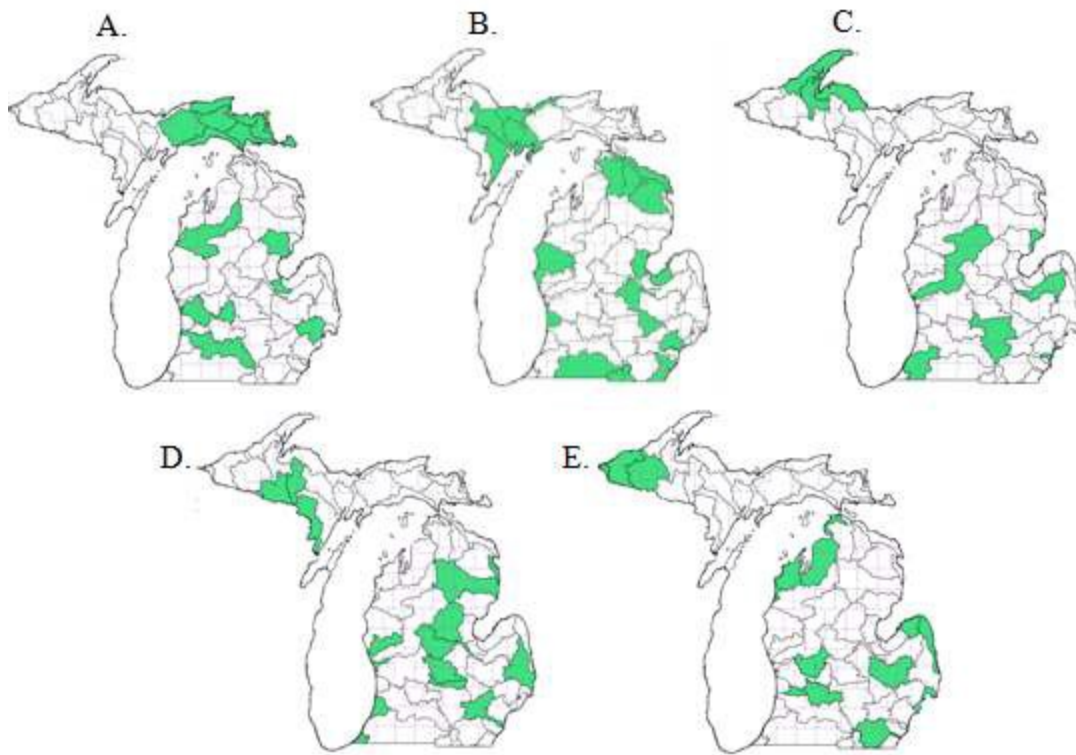


Figure 2. Michigan's watershed sampling units (shaded per year). A. 1999, 2004, 2008; B. 2000, 2005, 2009; C. 2001, 2006, 2010; D. 2002, 2007, 2011; and E. 2003, 2008, 2012.

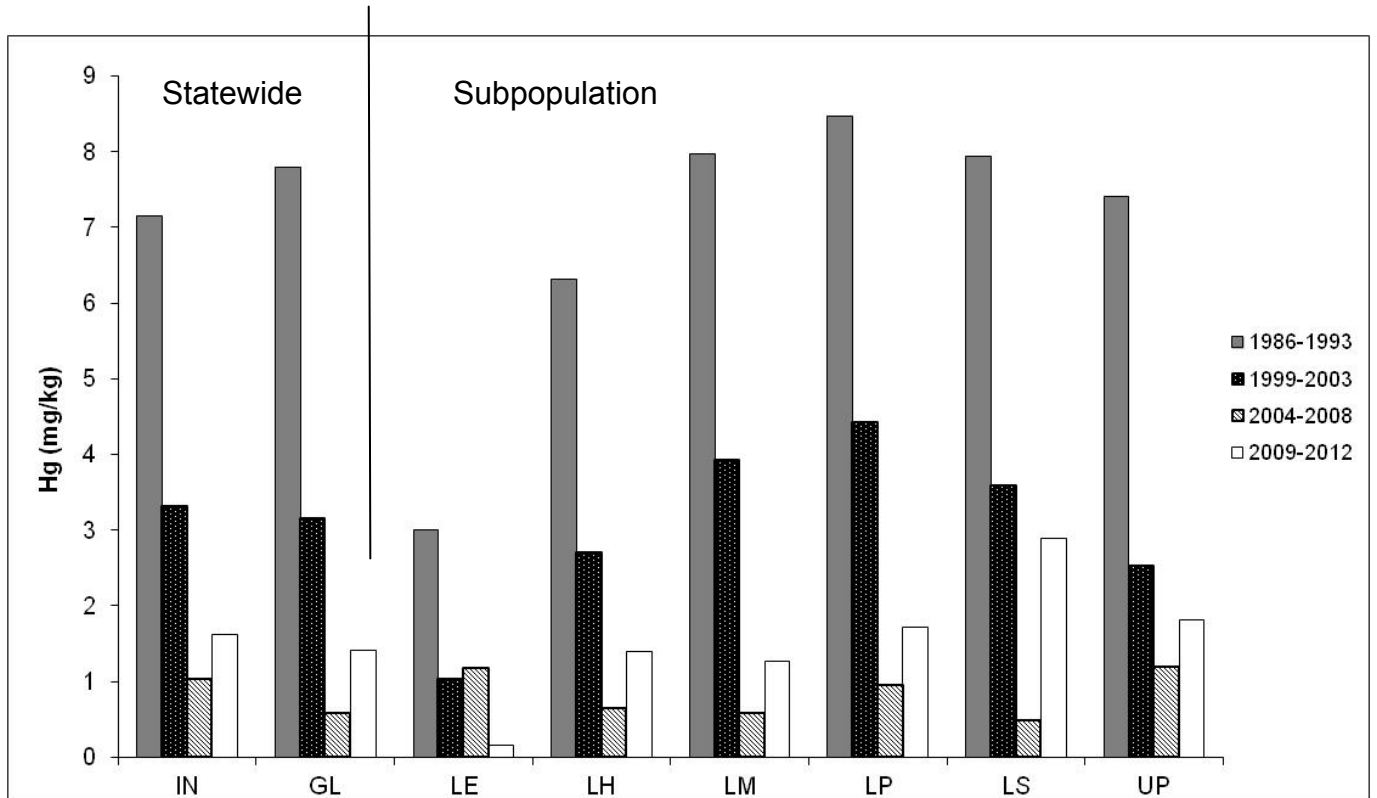


Figure 3. Geometric mean Hg concentrations in nestling bald eagles feathers in the Statewide and Subpopulation spatial scales for the following time periods: 1986-1993; 1999-2003, 2004-2008, and 2009-2012.

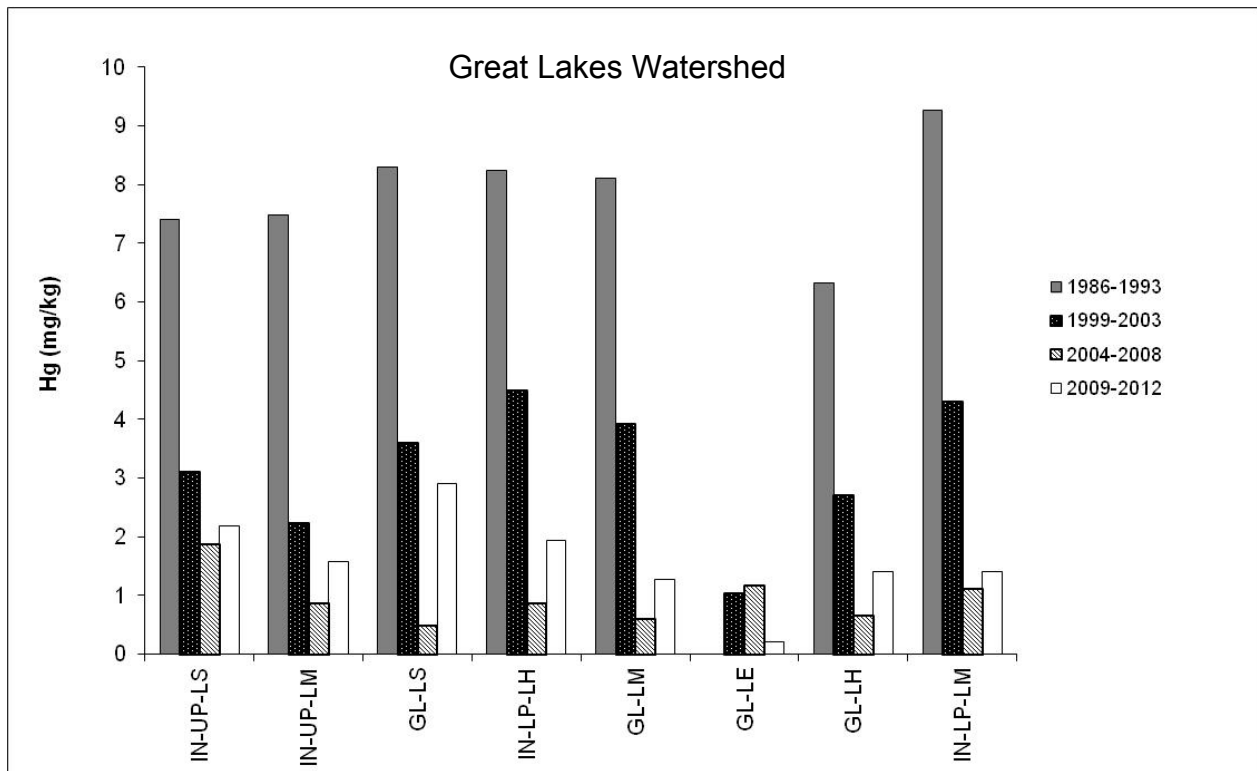


Figure 4. Geometric mean Hg concentrations in nestling bald eagle feathers in the Great Lakes watershed spatial scale for the following time periods: 1986-1993; 1999-2003, 2004-2008, and 2009-2012.