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Ecotoxicology

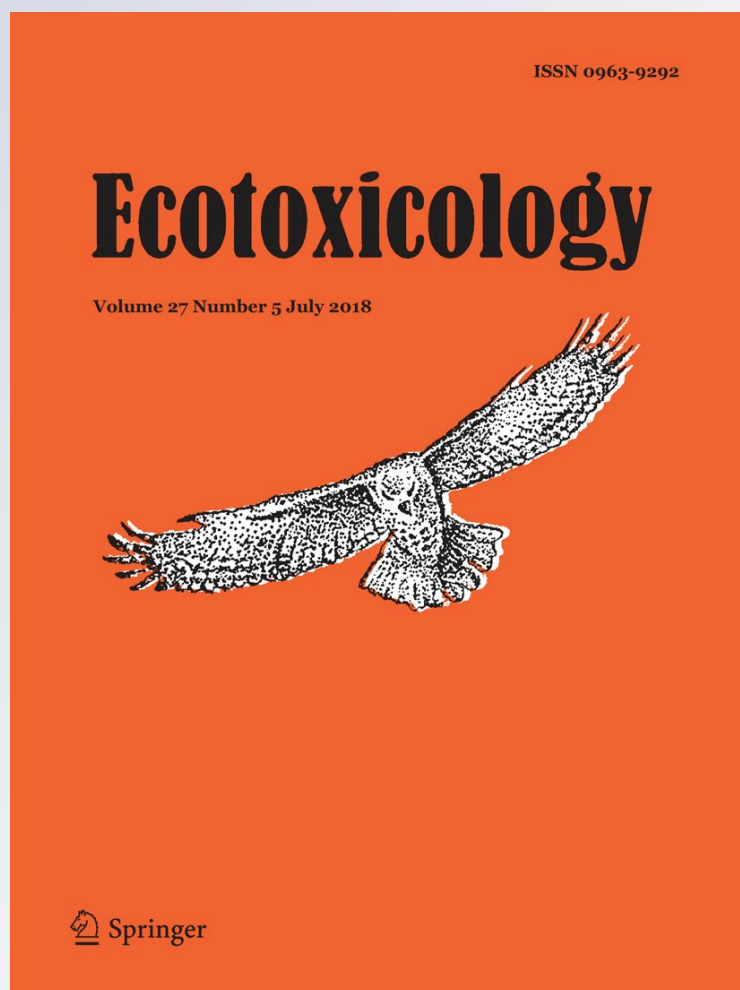
ISSN 0963-9292

Volume 27

Number 5

Ecotoxicology (2018) 27:605-618

DOI 10.1007/s10646-018-1933-5



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Patterns and trends in lead (Pb) concentrations in bald eagle (*Haliaeetus leucocephalus*) nestlings from the western Great Lakes region

Jason E. Bruggeman¹ · William T. Route² · Patrick T. Redig³ · Rebecca L. Key²

Accepted: 26 March 2018 / Published online: 10 April 2018
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Abstract

Most studies examining bald eagle (*Haliaeetus leucocephalus*) exposure to lead (Pb) have focused on adults that ingested spent Pb ammunition during the fall hunting season, often at clinical or lethal levels. We sampled live bald eagle nestlings along waterbodies to quantify Pb concentrations in 3 national park units and 2 nearby study areas in the western Great Lakes region. We collected 367 bald eagle nestling feather samples over 8 years during spring 2006–2015 and 188 whole blood samples over 4 years during spring 2010–2015. We used Tobit regression models to quantify relationships between Pb concentrations in nestling feathers and blood using study area, year, and nestling attributes as covariates. Pb in nestling feather samples decreased from 2006 to 2015, but there was no trend for Pb in blood samples. Pb concentrations in nestling feather and blood samples were significantly higher in study areas located closer to and within urban areas. Pb in feather and blood samples from the same nestling was positively correlated. Pb in feathers increased with nestling age, but this relationship was not observed for blood. Our results reflect how Pb accumulates in tissues as nestlings grow, with Pb in feathers and blood indexing exposure during feather development and before sampling, respectively. Some nestlings had Pb concentrations in blood that suggested a greater risk to sublethal effects from Pb exposure. Our data provides baselines for Pb concentrations in feathers and blood of nestling bald eagles from a variety of waterbody types spanning remote, lightly populated, and human-dominated landscapes.

Key words Bald eagle nestlings · Blood and feather samples · Censored data · *Haliaeetus leucocephalus* · Lead (Pb) pollution · National Park Service

Introduction

Bald eagles (*Haliaeetus leucocephalus*) can be valuable for assessing water quality due to their position as top predators of the aquatic food web (Stalmaster 1987), which makes them prone to accumulating high levels of some contaminants (e.g., Burger and Gotchfeld 2009; Elliott et al. 2009). Bald eagles have been the focus of many studies examining contaminants and their effects since bald eagle populations throughout North America underwent dramatic declines during the 1950s through 1970s due, in part, to negative effects of organochlorine pesticides (e.g., DDT) on reproductive success (Grier 1982; Stalmaster 1987; Elliott and Harris 2001). Contaminants documented in samples taken from live bald eagles are numerous and include: arsenic (Burger and Gotchfeld 2009); mercury (Wiemeyer et al. 1989; Wood et al. 1996; Dykstra et al. 2010); perfluorinated compounds (PFCs; Route et al. 2014b);

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10646-018-1933-5>) contains supplementary material, which is available to authorized users.

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polybrominated diphenyl ethers (PBDEs) and emerging flame retardants (Venier et al. 2010; Route et al. 2014a); and organochlorine pesticides and polychlorinated biphenyls (PCBs; Wiemeyer et al. 1989; Dykstra et al. 2010; Pittman et al. 2015). At sufficiently high levels, these pollutants can have varying impacts on bald eagles and other birds, including depressed reproductive rates, behavioral changes, altered brain chemistry, and mortality (Scheuhammer 1987; Dykstra et al. 2001, 2005; Rutkiewicz et al. 2011).

Although bald eagles primarily feed on fish and other aquatic species, they will also opportunistically scavenge on hunter- and car-killed ungulates and other carrion and prey on terrestrial wildlife (Dunstan and Harper 1975; Livingston et al. 1990; Kozie and Anderson 1991; Watson et al. 1991). The propensity of bald eagles to feed on remains of hunter-killed animals also makes them useful for assessing the uptake of contaminants, most notably lead (Pb) from spent Pb ammunition, in terrestrial systems (e.g., Fisher et al. 2006; Cruz et al. 2012; Hunt 2012; Warner et al. 2014). Pb is a particularly toxic pollutant that can enter the environment from multiple sources in addition to spent ammunition, including aircraft, automobiles, metal industries, mining, and refineries (Beyer et al. 1985; Cade 2007; Tsipoura et al. 2011; U.S. Environmental Protection Agency [USEPA] 2013). The use of Pb fishing tackle by anglers also provides a means for bald eagles and other scavengers to ingest Pb directly through consumption of dead or dying fish entangled with Pb sinkers (Scheuhammer and Norris 1996). Exposure of humans and wildlife to Pb at sufficient levels may result in deleterious effects on behavioral, immunological, and neurological systems, and impair development (Burger 1995; Burger and Gochfeld 2000; USEPA 2013). Pb previously documented in bald eagles has generally been from incidents of direct, and often lethal, exposure in adults (Wiemeyer et al. 1989; Kramer and Redig 1997; Hunt 2012; Warner et al. 2014).

The influence of Pb on bald eagle nestlings has received much less attention than Pb poisoning in adults and studies reporting Pb concentrations in live nestlings are limited (e.g., Anthony et al. 1993; Burger and Gochfeld 2009). Bald eagle nestlings are born in spring and well after the fall hunting season for ungulates and upland game, thereby making it less likely that nestlings are fed carcasses with spent Pb ammunition. Therefore, the more likely pathways for Pb to enter the bloodstream of nestlings are through indirect atmospheric sources, ingesting prey brought back to the nest containing Pb fishing tackle, or eating other prey with a high body burden of Pb (e.g., upland game with Pb embedded in the tissue). Additionally, when bald eagles are nesting they are territorial and often feed themselves and their nestlings from prey caught or scavenged within their

territory (Stalmaster 1987), thus making nestlings a good indicator of local Pb contamination.

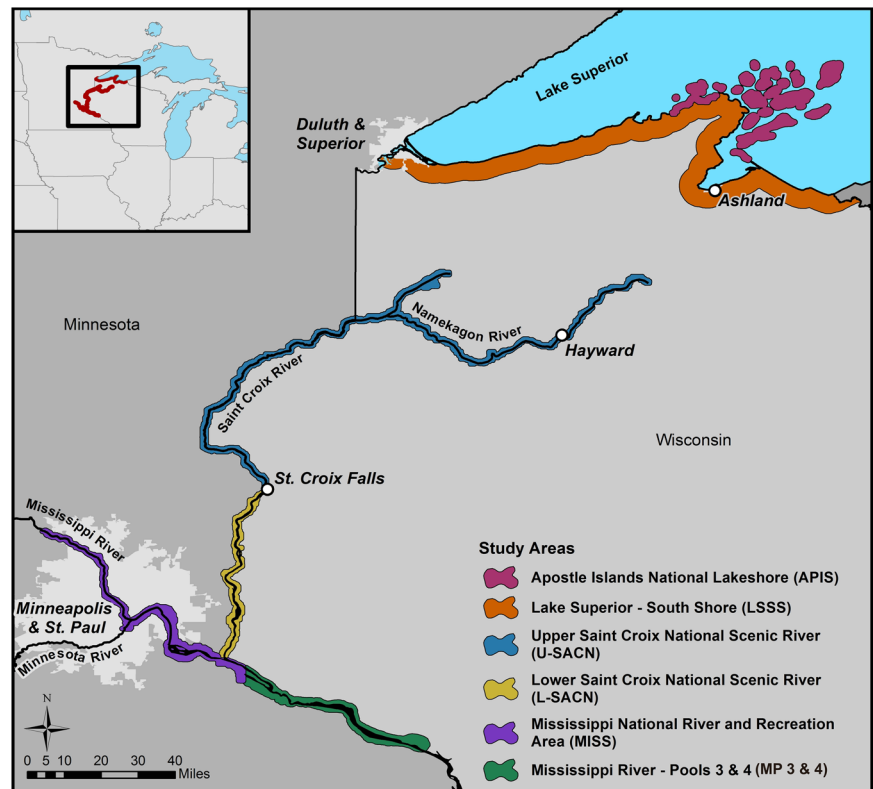
In 2006, the National Park Service (NPS) Great Lakes Inventory and Monitoring Network initiated an effort to record bald eagle productivity and monitor nestlings for a suite of contaminants, including Pb, to provide an index of waterbody and ecosystem health in 3 parks in the western Great Lakes region (Route and Elias 2007; Route et al. 2009). Work to date has documented spatial variability and trends in concentrations of DDE, PCBs, PFCs, PBDEs, and mercury in bald eagle nestlings throughout the region (Dykstra et al. 2010; Route et al. 2014a, b). The primary objective of this study was to assess spatial and temporal variation in concentrations of Pb in bald eagle nestlings from 3 national park units throughout the western Great Lakes region. To put results from these parks in context, we also quantified Pb concentrations in bald eagle nestlings from 2 study areas adjacent to the park units. Secondary objectives of our study included determining whether correlation existed between Pb concentrations in feather and blood samples taken from the same nestling, and samples from siblings in the same nest. Our study areas provided contrasts in surrounding land-use practices, proximity to cities and industries that were potential sources of Pb, and waterbody types (streams; large rivers; Laurentian Great Lakes). Therefore, we hypothesized Pb concentrations would be higher in nestlings located in study areas or along waterbodies that were closer to urban areas. To our knowledge, our study provides the largest sampling effort of live bald eagle nestlings to document patterns and trends in Pb.

Methods

Study areas

Our study areas consisted of 3 national park units and land along 2 nearby rivers and lakes in Minnesota and Wisconsin, USA (Fig. 1). We measured concentrations of Pb in bald eagle nestlings from the Apostle Islands National Lakeshore (APIS), Mississippi National River and Recreation Area (MISS), and St. Croix National Scenic Riverway (SACN). We divided SACN into upper (U-SACN) and lower (L-SACN) study areas due to differences in hydrology and surrounding land-use practices (Dykstra et al. 2010). When time and funding allowed, we collaborated with Wisconsin Department of Natural Resources and partners to sample bald eagle nestlings from Wisconsin's Lake Superior South Shore (LSSS) and the Minnesota Pollution Control Agency to sample downstream from MISS in Pools 3 and 4 of the Mississippi River (MP 3 & 4).

Fig. 1 Location of study areas in Minnesota and Wisconsin, USA, where bald eagle nestlings were sampled for Pb between 2006–2015



Data collection

We used aircraft to identify all occupied and productive bald eagle nests in each of the study areas (Fig. 1). From mid-May to late June during 2006–2011 and 2014–2015, we climbed to productive nests, counted the number of nestlings, hand-captured nestlings, and lowered them to the ground for sampling. We attempted to sample all nests in a given study area, excluding those that were unsafe to climb, had nestling(s) too old or young to sample, or when we were denied access through private property. We weighed, measured, and gave each nestling a general examination, and determined nestling age from the length of the 8th primary wing feather and sex by PCR-based genetic analysis. Two to 4 breast feathers comprised the primary sample for Pb exposure in all years. We randomly selected 1 nestling from each nest as the study bird and its feather samples were analyzed within 8 months. We archived feather samples from remaining siblings in individual sealed envelopes at room temperature for later analysis. Routine quality control procedures in the laboratory (see below) included duplicate samples to measure precision. Due to the small mass of breast feathers, duplicate samples consisted of 2 individual breast feathers plucked from different locations on the same nestling. These duplicates served both as a quality assurance measure and provided an opportunity to assess the variability of Pb accumulation between breast

feathers on the same nestling. In 2010, 2011, 2014, and 2015, we also extracted 1.0 mL of whole blood from the brachial vein of nestlings using a sterile, polypropylene syringe, and 22-gauge needle. The blood sample was immediately transferred to a vacutainer containing 7.5% EDTA and gently mixed. We froze blood samples within 8 h at -20°C until they were analyzed within 8 months.

Laboratory analyses

We sent bald eagle feathers and whole blood samples to the Wisconsin State Laboratory of Hygiene (Madison, Wisconsin, USA) for analysis of total Pb. The limit of detection (LOD) for Pb concentrations in feathers ranged between $0.067\text{--}0.552\text{ }\mu\text{g/g}$ (wet weight) and between $0.20\text{--}0.60\text{ }\mu\text{g/dL}$ (wet weight) for Pb concentrations in whole blood depending on the year samples were analyzed. In the lab, 1 whole nestling breast feather (measured to nearest 0.0001 g), was placed in a vial with 1.0 mL of concentrated nitric acid. Vials were capped and shaken, and samples were allowed to pre-digest overnight. Vials were then placed into a hot block digestion system at 95°C for 1 h. After cooling, 0.5 mL of hydrogen peroxide was added to each sample and the vials were re-heated to 95°C for 1/2-h. The addition of hydrogen peroxide and heat was repeated and the cooled solutions were brought to a final volume of 25 mL with ultrapure water. Each digestion batch of 20

contained a blank, a lab-fortified blank, and a control with certified reference material (NIST TORT-2); 10% of samples were analyzed in duplicate and spiked. The fortified blank was analyzed first and Pb concentration confirmed to within 15% of the true value before samples were analyzed. Digested solutions were analyzed for total Pb by inductively coupled plasma-mass spectroscopy according to USEPA Method 1638 (USEPA 1996). The instrument was calibrated with a blank and 5 standards prepared from an internal standard mix of gallium, indium, and bismuth. Calibration was verified initially and again after every tenth sample with a blank and a quality control sample from a source that differed from the standards. We note that feathers were not cleaned before analysis and it is possible that Pb from exogenous sources was included on feathers. Nestlings begin developing breast feathers at 3–4 weeks of age and all of the nestlings sampled were ≤ 10 weeks old, so measurements of Pb in feathers included internal Pb exposure and up to 6 weeks of exogenous exposure.

Statistical analyses

Pb in feather and whole blood samples from bald eagle nestlings

We used nonparametric Kaplan-Meier methods using function CENFIT from package NADA (Helsel 2012; Lee 2015) in R version 3.1.3 (R Core Team 2015) to calculate medians and standard deviations of Pb concentrations in nestling feather and whole blood samples. Based on recommendations summarized in Helsel (2012), Kaplan-Meier methods were suitable for calculating summary statistics for our dataset that consisted of $< 50\%$ of observations with Pb concentrations $< \text{LODs}$. Although Kaplan-Meier methods are typically used for survival analysis of right-censored data and our data consisted of left-censored data with a lower bound of 0, NADA “flips” left-censored data to treat them as right-censored, performs necessary calculations of the survival function probability as detailed in Helsel (2012), p 70–77, and then transforms estimates back to the appropriate scale (Helsel 2012). For CENFIT, we assigned censored data the corresponding LOD value with an indicator variable denoting the observation was $< \text{LOD}$, and uncensored data the actual Pb concentration value and an indicator variable denoting the observation was not censored.

We defined 6 covariates for use in modeling factors related to Pb concentrations in nestling feather and whole blood samples as 2 separate analyses: *age*, nestling age in days; *area*, categorical variable denoting the study area (APIS, L-SACN, LSSS, MISS, MP 3 & 4, U-SACN); *sex*, nestling male or female; *territory*, categorical variable for the nesting territory; *weight*, nestling weight (in kg); and

year, categorical variable denoting the year. We used the covariates to develop 23 Tobit regression models (Tobin 1958), which is a maximum likelihood estimation modeling approach appropriate for left-censored data (Helsel 2012). We specified a lognormal distribution in our models because of the skewed distribution of our data and lower bound of 0 (Helsel 2012). We did not include *weight* and *age* in models together because of collinearity, or a *year*area* interaction because of limited sample sizes for each study area per year. We included *territory* as a cluster term in each model to account for repeated observations from the same territories across years. We used function SURV within function SURVREG in R package SURVIVAL (R Core Team 2015; Therneau 2015) to separately fit our feather and whole blood models and estimate covariate coefficients for each analysis. In function SURV, we treated censored and uncensored data similar to CENFIT as described above. The likelihood function for Tobit models accounts for whether the observation is censored or not (Helsel 2012). We calculated a corrected Akaike's Information Criterion (AIC_c) value and Akaike weight (w) for each model, ranked models, and selected the best-approximating models with $\Delta\text{AIC}_c < 2$ for each analysis (Burnham and Anderson 2002). For each significant categorical covariate in the best-approximating models, we examined pairwise comparisons among all categories using the generalized Wilcoxon score test with function CENDIFF in NADA (Helsel 2012; Lee 2015) in R (R Core Team 2015). We determined if a significant difference existed between categories using Bonferroni's individual error rate of α/g , where α is the overall error rate of 0.05 and g is the number of possible pairwise comparisons among categories (Helsel 2012). We conducted a separate regression analysis using function LM in R (R Core Team 2015) to determine how *weight* of sampled nestlings was related to *sex* and *age*.

We analyzed the correlation between Pb concentrations in feather and blood samples obtained from the same nestling using Akritas-Theil-Sen (ATS) nonparametric regression (Akritas et al. 1995), which allows for analyzing doubly censored data in which x and y variables are both censored. Other regression methods for estimating the slope of censored data only allow the y variable to be censored (Helsel 2012). Use of ATS regression to estimate a slope assumes the data follow a linear pattern (Akritas et al. 1995). Because our data were not linear, we used a logarithmic transformation of Pb concentration in blood (Akritas et al. 1995). We also examined correlations between Pb concentrations in blood and feathers from siblings sampled from the same nest using ATS regression (Akritas et al. 1995) with a logarithmic transformation of Pb concentration for the first sibling to linearize the data. We analyzed the relationship between Pb concentrations in duplicate feather

samples from the same nestling using ATS regression and used parametric maximum likelihood estimation methods for censored data (Helsel 2012) with a lognormal distribution to determine whether significant differences existed between the means of the original and duplicate feather samples. We used function CENKEN in NADA (Helsel 2012; Lee 2015) for ATS regression to calculate an ATS slope, intercept, τ correlation coefficient, and P -value. We used function CENMLE in NADA (Helsel 2012; Lee 2015) for the parametric maximum likelihood estimation. For each analysis, we assigned censored data the corresponding LOD value with an indicator variable denoting the observation was $< \text{LOD}$, and uncensored data the actual Pb concentration value and an indicator variable denoting the observation was not censored.

Throughout all of our analyses, we did not directly substitute any value for samples with Pb concentrations $< \text{LOD}$. Substituting the LOD, one-half the LOD, or 0 for values of non-detects, or removing censored data from the analysis, are not considered appropriate methods because they can lead to biased results and erroneous findings (Helsel 2006; 2012).

Number of nestlings in productive bald eagle nests

We used mixed-effects Poisson regression (Zuur et al. 2009) to model the relationship between the number of nestlings counted in productive nests during ground surveys and *area*, *year*, and *area + year*, treating *territory* as a random intercept effect in each model and *area* and *year* as categorical covariates. For this analysis we excluded failed nests. We used function GLMER in R package LME4 (Bates et al. 2015; R Core Team 2015) to fit 3 models and estimate covariate coefficients. We calculated an AIC_c value for each model, selected the model(s) with $\Delta\text{AIC}_c < 2$, and calculated an overdispersion parameter (ϕ) for each best-approximating model as $\phi = D / (n - p)$, where D is the deviance with $n - p$ degrees of freedom (Zuur et al. 2009).

Results

Pb concentrations in feather and whole blood samples from bald eagle nestlings

Feathers

We obtained feather samples from 367 nestlings in 153 unique territories between 2006–2015 in 6 study areas (Tables 1, 2; Fig. 2a; Online Resource 1, Table S1). Nestling age ranged from 23–71 days (mean = 42.5; SE = 0.455), weight ranged from 1.8–5.5 kg (mean = 3.48; SE =

0.035), and 192 (52%) of the nestlings sampled were female. Of the 367 samples, 166 (45%) had Pb concentrations $< \text{LOD}$ (Table 1). Pb concentrations in the remaining 201 samples ranged from 0.140–3.71 $\mu\text{g/g}$ wet weight (Kaplan-Meier median = 0.163; Table 1) with 5 and 10 samples above the 97.5th ($\geq 1.55 \mu\text{g/g}$) and 95th percentiles ($\geq 1.01 \mu\text{g/g}$), respectively. There were 2 best-approximating models of Pb concentrations in nestling feathers with $\Delta\text{AIC}_c < 2$ (Table 3; Online Resource 1, Table S2). The model with the most support had $w = 0.671$ and included *area*, *year*, and *age*; the model with the second-most support ($w = 0.268$) included these 3 covariates and *sex*, although covariate coefficients for *sex* were centered on zero (Table 3). Pb concentrations in feathers were significantly higher in L-SACN, MISS, and MP 3 & 4 relative to APIS; and MISS and MP 3 & 4 relative to U-SACN (Table 3, Fig. 3a; Online Resource 2, Figs. S1–S5). There was no difference between Pb concentrations in feathers from LSSS and all other areas. Pb concentrations in feathers decreased over time: concentrations were significantly higher in 2006 compared to 2007–2011 and 2014–2015; 2008 and 2009 concentrations were significantly higher than those from 2011; and 2007–2009 concentrations were significantly higher than those from 2014 (Table 3; Fig. 3b). Pb concentrations in feathers were positively correlated with nestling age (Table 3; Fig. 4a). The *weight* of female nestlings was significantly higher than males ($P < 0.001$) and positively correlated with *age* ($P < 0.001$) as: $\text{weight} = 1.67 - 0.657 * \text{sex}(\text{male}) + 0.050 * \text{age}$.

We obtained duplicate feather samples from 66 individual nestlings during 2006–2015. There was a significant, positive relationship between Pb concentrations in original and duplicate feather samples (ATS regression slope = 1.26; intercept = -0.083 ; $\tau = 0.478$; $P < 0.001$; Online Resource 3, Fig. S11). There was no difference between the mean Pb concentrations from original and duplicate feather samples ($P = 0.94$).

Whole blood

We obtained whole blood samples from 188 nestlings in 109 unique territories between 2010–2015 in the 6 study areas (Tables 1, 2; Fig. 2b). Nestling age ranged from 19–69 days (mean = 44.0; SE = 0.636), weight ranged from 1.8–5.5 kg (mean = 3.57; SE = 0.048), and 102 (54%) of nestlings sampled were female. Of the 188 samples, 80 (43%) had Pb concentrations $< \text{LOD}$ (Table 1). Pb concentrations in the remaining 108 samples ranged from 0.200–26.4 $\mu\text{g/dL}$ wet weight (Kaplan-Meier median = 0.370; Table 1) with 3 and 6 samples above the 97.5th ($> 11.7 \mu\text{g/dL}$) and 95th percentiles ($> 8.2 \mu\text{g/dL}$), respectively. We used data from 4 of the 6 study areas

Table 1 Total number of samples (n), number of samples with Pb concentrations < limit of detection (LOD) (n_{censored}), and Kaplan-Meier median and standard deviation for Pb concentrations in bald eagle nestling feathers collected between 2006–2015 and whole blood samples collected between 2010–2015 at 6 study areas: Apostle Islands National Lakeshore (APIS); Lower Saint Croix National Scenic River (L-SACN); Lake Superior South Shore (LSSS); Mississippi National River and Recreation Area (MISS); Mississippi River Pools 3 & 4 (MP 3 & 4); Upper Saint Croix National Scenic River (U-SACN)

Study area	Feather samples				Whole blood samples			
	n	n_{censored}	Median ^a (μg/g)	Standard deviation	n	n_{censored}	Median ^a (μg/dL)	Standard deviation
APIS	57	43	<0.067	0.116	35	14	0.260	2.33
L-SACN	64	26	0.150	0.332	35	19	0.250	3.30
LSSS ^b	11	6	<0.067	0.099	1	1	<0.60	n/a
MISS	139	40	0.247	0.498	77	19	0.560	3.60
MP 3 & 4 ^b	31	6	0.190	0.104	2	2	<0.60	n/a
U-SACN	65	45	<0.067	0.115	38	25	<0.60	2.12

^aMedians for each study area < LOD are reported as < 0.067 for feather samples and < 0.60 for whole blood samples

^bAll blood samples from LSSS and MP 3 & 4 had Pb concentrations < LODs

Table 2 Yearly total number of samples (n), number of samples with Pb concentrations < limit of detection (LOD) (n_{censored}), and Kaplan-Meier median and standard deviation for Pb concentrations in bald eagle nestling feathers collected between 2006–2015 and whole blood samples collected between 2010–2015 in 6 study areas in Minnesota and Wisconsin

Year	Feather Samples				Whole Blood Samples			
	n	n_{censored}	Median ^a (μg/g)	Standard deviation	n	n_{censored}	Median ^a (μg/dL)	Standard deviation
2006	32	0	0.370	0.753	n/a ^b	n/a	n/a	n/a
2007	35	15	0.170	0.436	n/a	n/a	n/a	n/a
2008	42	12	0.200	0.199	n/a	n/a	n/a	n/a
2009	39	9	0.200	0.491	n/a	n/a	n/a	n/a
2010	60	30	<0.067	0.135	28	13	0.320	3.39
2011	55	31	<0.067	0.112	56	36	<0.60	1.60
2014	49	35	<0.067	0.090	49	23	0.360	2.11
2015	55	34	0.146	0.233	55	8	0.380	4.30

^aMedians for each year < LOD are reported as < 0.067 for feather samples and < 0.60 for whole blood samples

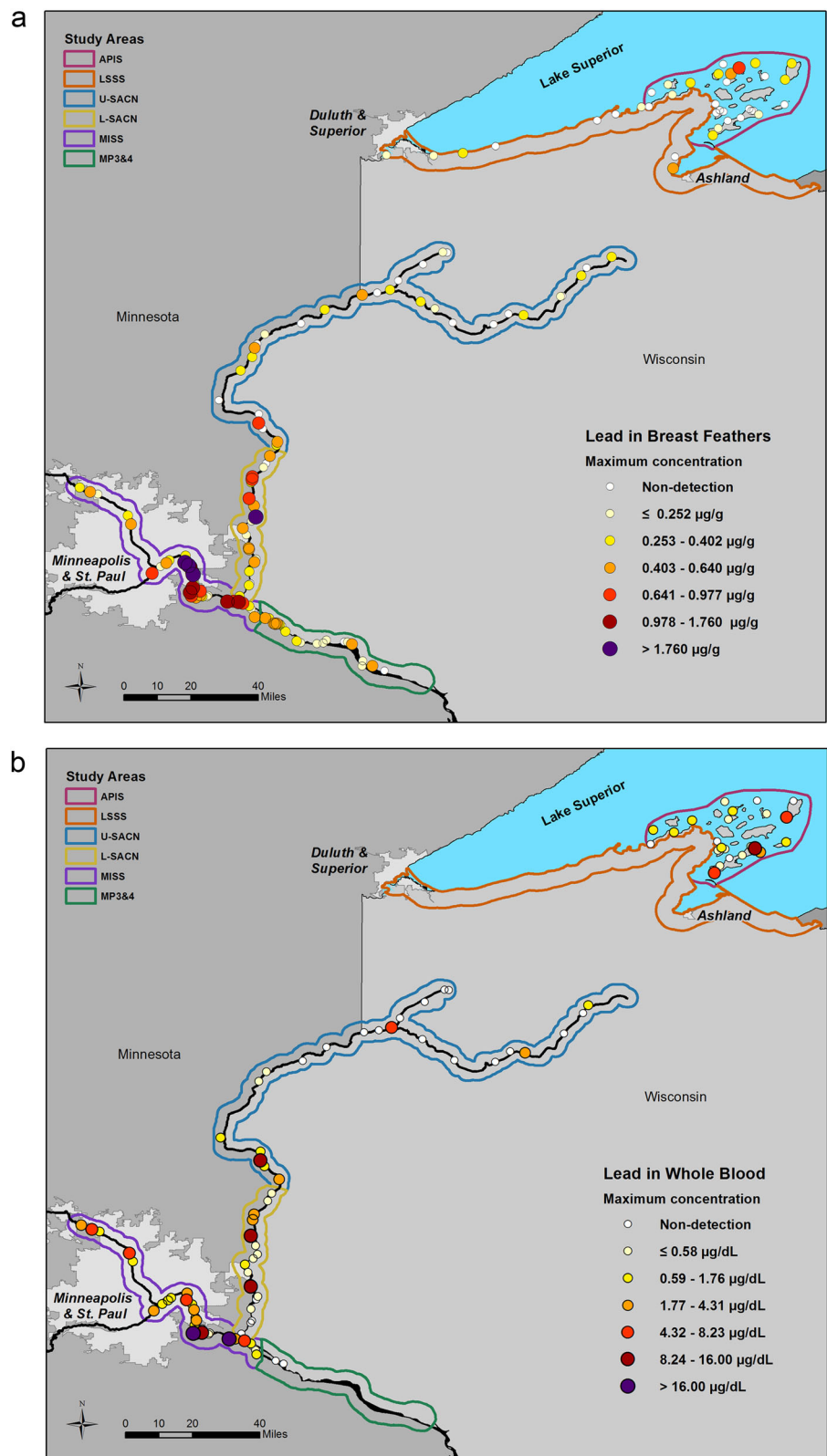
^bWhole blood samples were not collected during 2006–2009

(185 samples) for our modeling analysis, omitting LSSS and MP 3 & 4 due to small sample sizes. There were 3 models of Pb concentrations in nestling blood with $\Delta\text{AIC}_c < 2$ (Table 4; Online Resource 1, Table S3). Best-approximating models included *area*, *sex*, *age*, and *weight* covariates, but not *year* (Table 4). Pb concentrations in blood samples from MISS were significantly higher than those from L-SACN and U-SACN, and higher than those from APIS, but this difference was not statistically significant ($P = 0.055$; Table 4; Online Resource 2, Figs. S6–10). Pb in samples from L-SACN and U-SACN were not significantly different than samples from APIS or from each other. Pb concentrations in blood were significantly higher in male nestlings, but *age* and *weight* were not statistically significant covariates (Table 4).

Paired feather and whole blood samples from individuals and siblings

We obtained paired feather and blood samples from 187 individual nestlings during 2010–2015. There was a significant, positive relationship between Pb concentrations in feather samples and those in whole blood samples from the same individual (ATS regression slope = 0.146; intercept = 0.223; $\tau = 0.237$; $P < 0.001$; Fig. 4b). We collected blood samples from 61 pairs of siblings (i.e., nest mates) in 2014–2015 and there was a significant, positive relationship between Pb concentrations in blood samples of siblings (ATS regression slope = 0.605; intercept = 1.11; $\tau = 0.438$; $P < 0.001$; Online Resource 3, Fig. S12a). We also collected feather samples from 272 pairs of siblings during 2006–2015 and found a

Fig. 2 Spatial variability in the maximum concentration of Pb in samples of **a** feathers from bald eagle nestlings, and **b** whole blood from bald eagle nestlings. Feather samples ($n = 367$) were obtained between 2006–2015, and whole blood samples ($n = 188$) were obtained during 2010–2015 from 6 study areas in Minnesota and Wisconsin: Apostle Islands National Lakeshore (APIS); Lower Saint Croix National Scenic River (L-SACN); Lake Superior-South Shore (LSSS); Mississippi National River and Recreation Area (MISS); Mississippi River Pools 3 & 4 (MP 3 & 4); Upper Saint Croix National Scenic River (U-SACN). The maximum concentration is the highest concentration found in each bald eagle territory during the study



significant, positive relationship between Pb concentrations in feather samples of siblings (ATS regression slope = 0.116; intercept = 0.433; $\tau = 0.298$; $P < 0.001$; Online Resource 3,

Fig. S12b). Data for siblings that were not part of the original random sampling selection were used only for these comparisons and not the modeling analyses detailed above.

Table 3 Coefficient estimates (β) and standard errors (SE) for covariates included in the 2 best-approximating Tobit regression models with $\Delta AIC_c < 2$ from the analysis relating factors to Pb concentrations in bald eagle nestling feathers obtained between 2006–2015 from 6 study areas in Minnesota and Wisconsin. Covariates are defined in the text; n/a denotes a covariate that was not included in model. A table of model results is in Online Resource 1, Table S2

Covariate	$\Delta AIC_c = 0.00$; $w = 0.671$	$\Delta AIC_c = 1.84$; $w = 0.268$
	β (SE)	β (SE)
Intercept ^a	-2.19 (0.339) ^b	-2.25 (0.346) ^b
area = L-SACN	0.872 (0.173) ^b	0.866 (0.173) ^b
area = LSSS	0.080 (0.292)	0.072 (0.293)
area = MISS	1.27 (0.160) ^b	1.26 (0.160) ^b
area = MP 3 & 4	0.925 (0.183) ^b	0.926 (0.182) ^b
area = U-SACN	-0.011 (0.202)	-0.015 (0.203)
year = 2007	-0.810 (0.189) ^b	-0.799 (0.190) ^b
year = 2008	-1.03 (0.197) ^b	-1.01 (0.196)
year = 2009	-1.18 (0.227) ^b	-1.16 (0.226) ^b
year = 2010	-1.42 (0.186) ^b	-1.41 (0.184) ^b
year = 2011	-1.60 (0.170) ^b	-1.59 (0.170) ^b
year = 2014	-2.27 (0.229) ^b	-2.27 (0.228) ^b
year = 2015	-1.48 (0.183) ^b	-1.47 (0.182) ^b
age	0.020 (0.006) ^b	0.021 (0.006) ^b
sex = male	n/a	0.054 (0.087)

Study areas are: *APIS* Apostle Islands National Lakeshore, *L-SACN* Lower Saint Croix National Scenic River, *LSSS* Lake Superior South Shore, *MISS* Mississippi National River and Recreation Area, *MP 3 & 4* Mississippi River Pools 3 & 4, *U-SACN* Upper Saint Croix National Scenic River

^aIntercept includes area = APIS, sex = female, and year = 2006 covariates

^bEstimates are statistically significant at $\alpha = 0.05$

Number of nestlings in productive bald eagle nests

We counted the number of nestlings from 367 productive bald eagle nests representing 152 unique territories during 2006–2015. Number of nestlings in the 367 productive nests ranged from 1–4 young (mean = 1.88; SE = 0.036). There was one best-approximating model that contained area, had $w = 0.995$, and $\phi = 2.8$, suggesting slight overdispersion than expected from a Poisson distribution. Number of nestlings per productive nest in MISS was significantly higher than LSSS and U-SACN and higher than APIS, although the lower 95% confidence interval for MISS slightly overlapped the APIS productivity estimate (Fig. 5a). Number of nestlings per productive nest was significantly higher in L-SACN and MP 3 & 4 compared to U-SACN (Fig. 5a). Number of nestlings per productive nest was significantly higher in 2009 compared to 2014, but did not significantly differ among all other years (Fig. 5b).

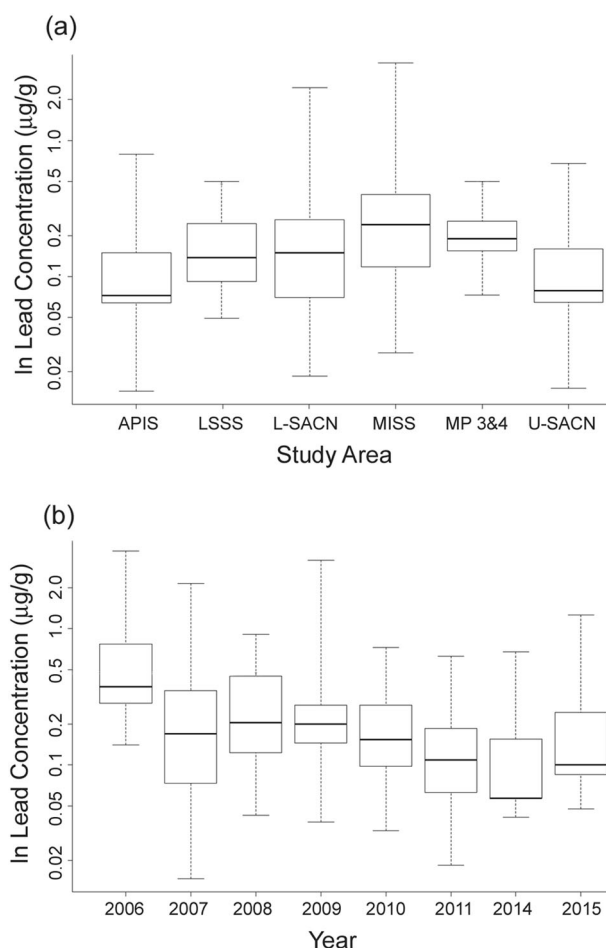


Fig. 3 Variability in the natural logarithm of Pb concentrations ($\mu\text{g/g}$) in bald eagle nestling feather samples by (a) study area, and (b) year. Feather samples ($n = 367$) were obtained between 2006–2015 from 6 study areas in Minnesota and Wisconsin: Apostle Islands National Lakeshore (APIS); Lower Saint Croix National Scenic River (L-SACN); Lake Superior-South Shore (LSSS); Mississippi National River and Recreation Area (MISS); Mississippi River Pools 3 & 4 (MP 3 & 4); Upper Saint Croix National Scenic River (U-SACN). Boxplots depict the 25th, 50th, and 75th percentiles of the data. Sample sizes for each study area and year were: APIS, $n = 57$; L-SACN, $n = 64$; LSSS, $n = 11$; MISS, $n = 139$; MP 3 & 4, $n = 31$; U-SACN, $n = 65$; 2006, $n = 32$; 2007, $n = 35$; 2008, $n = 42$; 2009, $n = 39$; 2010, $n = 60$; 2011, $n = 55$; 2012, $n = 0$; 2013, $n = 0$; 2014, $n = 49$; 2015, $n = 55$

Discussion

Collection of avian feather and blood samples has provided valuable insight into the pervasiveness of heavy metal pollutants in the environment (Rattner et al. 2008; Eulares et al. 2011; Meyer et al. 2013; Pittman et al. 2011; Jackson et al. 2015). Our study illustrates the value of collecting samples from bald eagle nestlings as an index of local Pb contamination and documents the prevalence and distribution of Pb pollution in aquatic systems of the western Great Lakes region. We observed a decreasing trend in Pb in nestling breast feathers over 8 years from 2006 through

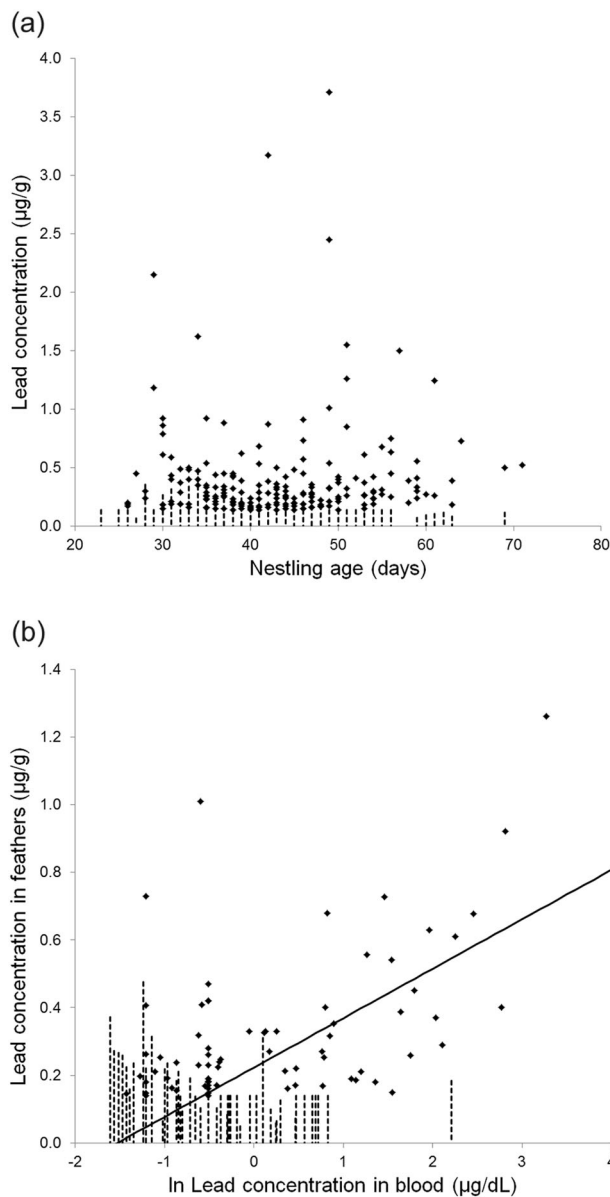


Fig. 4 Relationships between **a** Pb concentration (µg/g) in bald eagle nestling feathers and nestling age (days), and **b** Pb concentration in bald eagle nestling feathers (µg/g) and the natural logarithm of Pb concentration in nestling blood (µg/dL). **a** Feather samples ($n = 367$) were obtained from 152 unique territories between 2006–2015 throughout 6 study areas in Minnesota and Wisconsin. **b** Feather and whole blood samples were obtained from 187 individual nestlings during 2010–2015 from 6 study areas in Minnesota and Wisconsin. The equation for the line of best fit for **(b)**, derived from Akritas-Teil-Sen nonparametric regression, relating Pb concentrations is $Pb_{\text{feather}} = 0.223 + 0.146 \ln(Pb_{\text{blood}})$, where Pb_{feather} and Pb_{blood} are the concentrations of Pb in feather and blood samples, respectively. Vertical dashed lines denote censored observations below the multiple limits of detection

2015 (Fig. 3b). This decrease coincides with USEPA data showing an 87% decrease in the national average of Pb concentrations in air from 2000 to 2014, which was largely attributed to regulations reducing Pb content in vehicle

Table 4 Coefficients (β) and standard errors (SE) for covariates included in the 3 best-approximating Tobit regression models with $\Delta AIC_c < 2$ from an analysis of Pb concentrations in bald eagle nestling whole blood samples collected between 2010–2015 from 4 study areas in Minnesota and Wisconsin. Covariates are defined in the text; n/a denotes a covariate that was not included in model. A table of model results is in Online Resource 1, Table S3

Covariate	$\Delta AIC_c = 0.00$; $w = 0.279$	$\Delta AIC_c = 0.47$; $w = 0.220$	$\Delta AIC_c = 1.91$; $w = 0.108$
	β (SE)	β (SE)	β (SE)
Intercept ^a	−1.28 (0.245) ^b	−2.01 (0.596) ^b	−0.886 (0.866)
area = L-SACN	−0.218 (0.389)	−0.279 (0.398)	−0.226 (0.385)
area = MISS	0.527 (0.274)	0.501 (0.286)	0.511 (0.267)
area = U-SACN	−0.308 (0.458)	−0.385 (0.455)	−0.319 (0.459)
sex = male	0.598 (0.220) ^b	0.634 (0.224) ^b	0.533 (0.275)
age	n/a	0.017 (0.013)	n/a
weight	n/a	n/a	−0.099 (0.218)

APIS Apostle Islands National Lakeshore, L-SACN Lower Saint Croix National Scenic River, MISS Mississippi National River and Recreation Area, U-SACN Upper Saint Croix National Scenic River

^aIntercept includes area = APIS and sex = female covariates

^bEstimates are statistically significant at $\alpha = 0.05$

gasoline (USEPA 2013; USEPA 2015). Reductions in other sources of Pb, such as banning of Pb shot for waterfowl hunting (Avery and Watson 2009) and removal of Pb from paint (Jacobs et al. 2002) likely contributed to the decrease as well. We also documented spatial patterns in Pb concentrations in bald eagle nestling feathers that are likely due to proximity to Pb sources and surrounding land-use practices (USEPA 2013).

For example, in the MISS study area that had some of the highest Pb concentrations in nestling feathers and whole blood (Fig. 3; Fig. S5; Fig. S10), many eagle territories are within metropolitan Minneapolis-St. Paul where they are exposed to effluent from municipal and industrial waste water treatment plants, as well as airborne pollutants from airports, refineries, power plants, and vehicle traffic, which all can be sources of Pb (USEPA 2013). Furthermore, bald eagles sampled from nests along the Mississippi River near and immediately downstream from Pig's Eye Lake had the highest Pb concentrations in nestling feathers (Fig. 2a; Fig. S5). Pig's Eye Lake is adjacent to both the outlet of the metropolitan waste water treatment plant and the former Pig's Eye Landfill, which is a Minnesota Superfund site (U. S. Department of Health and Human Services 2000). Soil samples from a battery disposal area in Pig's Eye Landfill and water samples from Battle Creek, which bisects the landfill and discharges into Pig's Eye Lake, documented elevated levels of Pb prior to the start of our study (U.S. Department of Health and Human Services 2000). Other

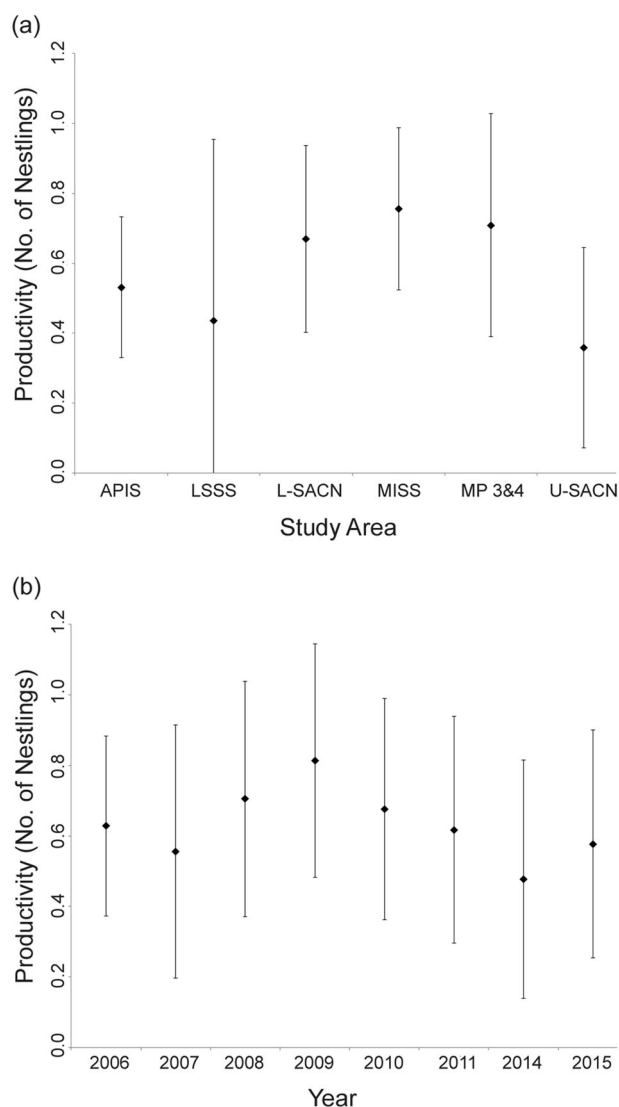


Fig. 5 Variability in model-estimated number of nestlings in productive bald eagle nests and 95% confidence intervals by **a** study area, and **b** year. Data were obtained from 367 productive bald eagle nests representing 152 unique territories between 2006–2015 from 6 study areas in Minnesota and Wisconsin: Apostle Islands National Lakeshore (APIS); Lower Saint Croix National Scenic River (L-SACN); Lake Superior-South Shore (LSSS); Mississippi National River and Recreation Area (MISS); Mississippi River Pools 3 & 4 (MP 3 & 4); Upper Saint Croix National Scenic River (U-SACN). Sample sizes for each study area and year were: APIS, $n = 57$; L-SACN, $n = 64$; LSSS, $n = 11$; MISS, $n = 139$; MP 3 & 4, $n = 31$; U-SACN, $n = 65$; 2006, $n = 32$; 2007, $n = 35$; 2008, $n = 42$; 2009, $n = 39$; 2010, $n = 60$; 2011, $n = 55$; 2012, $n = 0$; 2013, $n = 0$; 2014, $n = 49$; 2015, $n = 55$

researchers have also found elevated concentrations of heavy metals in wildlife near landfills (e.g., Arenal and Halbrook 1997; Sánchez-Chardi and Nadal 2007), and our results show Pb is accumulating in the food web and suggests chronic, localized contamination in the Pig's Eye Lake area of MISS.

Differences in waterbody type and size likely accounted for some of the spatial variability in concentrations of Pb.

Samples taken from nests along the Mississippi River, which is larger, slower-flowing, more productive, and in a more developed landscape, had significantly higher Pb concentrations than those taken from nests along the upper St. Croix River, which is smaller, faster-flowing, and in a less developed area. Likewise, nestling feathers sampled from APIS had lower Pb concentrations than those from all river-based study areas except U-SACN. In addition to having minimal human influence, nest sites located in the Apostle Islands National Lakeshore are surrounded by Lake Superior's cold, unproductive waters, which minimize Pb accumulation at higher trophic levels in the food web. Chen et al. (2000) found Pb concentrations were highest in eutrophic lakes in agricultural regions and bioaccumulation of Pb diminished with increasing trophic level, especially in cold water lakes.

Pb concentrations in feathers increased with nestling age, but we did not find this relationship for Pb in whole blood. Heavy metal accumulation in avian feathers has been related to concentrations in blood at the time of feather formation (Burger 1993; Furness 1993; Dauwe et al. 2003). Our results support the findings of others that Pb accumulates as nestlings grow and feathers develop; hence, Pb in feathers is a measure of accumulated exposure during the nestling's life. In contrast, Pb in nestling blood is primarily the result of exposure from consumption of Pb in a recent meal. Sources of this recent Pb exposure could include spent ammunition, fishing tackle, and prey that already contain a body burden of Pb in their tissue (Scheuhammer and Norris 1996; Fisher et al. 2006). We found correlations in Pb concentrations between siblings from the same nest in both feather and blood samples, suggesting siblings received similar exposures, which is in agreement with findings from studies on other bird species (e.g., Blanco et al. 2003).

Because we sampled nestlings during the late spring (May–June) and after the fall (September–November) hunting seasons for upland game and deer (*Odocoileus* spp.), it is unlikely nestlings ingested Pb bullet fragments from remains of legally hunted wild game, which is one of the most noted sources of adult bald eagle exposure and mortality from Pb (Hunt et al. 2006; Cruz et al. 2012; Franson and Russell 2014). We hypothesize the pathway of Pb exposure to bald eagle nestlings is primarily from feeding on fish or other prey that have a body burden of Pb in their tissue and/or ingesting Pb tackle attached to fish while attempting to eat them. For example, some of the exposures could have resulted from ingestion of small game (e.g., squirrels [*Sciurus* spp.], turkeys [*Meleagris gallopavo*]) or waterfowl containing Pb fragments or shot (Fallon et al. 2017). Through this study we found fish, bird, and mammal remains in 80, 30, and 25% of nests, respectively, and we found fishing tackle in 2% of nests (W. Route

unpublished data). The prevalence of fish in bald eagle diets illustrates their importance in assessing Pb contamination in aquatic systems.

The correlation we found between Pb concentrations in blood and feather samples from the same bald eagle nestling have implications for future sampling and monitoring protocols. Because blood and feather samples indicated different measures of Pb in a nestling, sampling efforts could be tailored depending on management questions. If there were concerns over recent Pb exposures, then collecting blood samples from nestlings would be best. However, if questions of accumulated Pb exposure were relevant, then collection of feather samples would be more appropriate.

We also found a correlation in Pb levels in feather and blood samples between siblings from the same nest, suggesting that random selection of 1 nestling is likely sufficient to obtain an index of Pb exposure to all nestlings in the nest. However, similar to Gangoso et al. (2009), we note significantly higher Pb levels in whole blood samples of males compared to females. There is evidence that Pb is deposited in bone at higher rates when birds are young, growing rapidly, and absorbing and depositing calcium into bone (Pain et al. 2009). Bald eagle nestling females grow faster and are larger, thus a higher proportion of Pb could be apportioned into bone rather than blood of females. Alternatively, female nestlings are more aggressive during feeding (Bortolotti 1986) and we found females were significantly heavier than males. We speculate that because adult bald eagles feed their young by stripping bits of flesh from prey, the more aggressive nestlings are first fed the outer portions of the carcass whereas the more passive nestlings may only eat the inner portions and entrails. Pb is found in higher concentrations in carcass entrails such as kidney and liver (Pain et al. 2009) and, therefore, less aggressive male nestlings may have higher blood Pb levels owing to the portion of the carcass they are more likely to be fed.

The number of nestlings in productive bald eagle nests suggests exposure to Pb in our study areas did not impair adult productivity at the broad spatial scales we evaluated. However, we only used data from productive nests (i.e., nests with ≥ 1 nestling) for the analysis, which does not account for nest failures. Number of nestlings in productive nests in MISS, which had some of the highest Pb concentrations in nestling feathers and whole blood, was significantly higher than LSSS and U-SACN and higher than APIS, although the latter was not statistically significant. Likewise, number of nestlings in productive nests was significantly higher in L-SACN and MP 3 & 4 compared to less-developed U-SACN. Companion studies on this same bald eagle population found no correlation between bald eagle reproductive rates and contemporary levels of mercury, DDE, PCBs, or perfluorinated chemicals in blood

samples (Dykstra et al. 2010; Route et al. 2014b). However, Rutkiewicz et al. (2011) found adult bald eagles in the Great Lakes region were exposed to mercury at levels capable of causing subclinical neurological changes that may negatively affect productivity with 14–27% of adult bald eagles at risk. Exposure to pollutants for bird species other than bald eagles has been shown to decrease reproductive rates or result in nest failure (Elliott et al. 1989; Steidl et al. 1991; Blus 2011; Harris and Elliott 2011; Rattner et al. 2011; Shore et al. 2011). Additional research would be needed to determine if nestlings demonstrate subclinical neurological or physiological changes as a result of the Pb concentrations found in this study.

The University of Minnesota Raptor Center considers adult bald eagles with blood Pb concentrations between 20–60 $\mu\text{g}/\text{dL}$ as having subclinical exposure and 61–120 $\mu\text{g}/\text{dL}$ as having clinical poisoning that is treatable through chelation therapy (Kramer and Redig 1997). One of the nestlings in our study was above the University of Minnesota Raptor Center's threshold for subclinical exposure with $>26 \mu\text{g}/\text{dL}$ and 2 nestlings had Pb levels between 16–17 $\mu\text{g}/\text{dL}$, which approached subclinical levels. Three of the nestlings had blood samples with Pb concentrations $\geq 16 \mu\text{g}/\text{dL}$, which was above the 97.5th percentile of our uncensored data. In comparison with other raptor species, Cade (2007) proposed a threshold for exposure of 10 $\mu\text{g}/\text{dL}$ of Pb in blood for California condors (*Gymnogyps californianus*), and Franson and Pain (2011) adopted subclinical and clinical poisoning ranges of 20–50 and 50–100 $\mu\text{g}/\text{dL}$, respectively, for Pb in blood of adult *Falconiforms*. More recently, Ecke et al. (2017) proposed a much lower sublethal concentration for golden eagles of 2.5 $\mu\text{g}/\text{dL}$, which they based on the Benchmark Dose Response of a 10% reduction in mean movement rate of radio-telemetered birds. These toxicological thresholds, however, are for adult birds and we are unaware of any toxicological studies or thresholds for Pb concentrations in bald eagle nestlings. Young, growing birds are more sensitive to toxic effects of heavy metals like Pb (Scheuhammer 1987). For example, Burger and Gochfeld (2005) found herring gull (*Larus argentatus*) chicks showed negative effects in thermoregulation, feeding, and other behaviors when dosed with Pb at levels that produced feather concentrations similar to that found in wild gulls (Burger and Gochfeld 1990). Exposure to Pb has resulted in reduced growth rates and lower fledging success for little blue heron chicks (*Egretta caerulea*) at Pb concentrations $<17 \mu\text{g}/\text{g}$ in feathers (Spahn and Sherry 1999). We found no effect of Pb on bald eagle productivity among our study areas, but we did not measure sublethal effects such as physiological or behavioral characteristics on individual nestlings.

Most research on sublethal thresholds and toxicological effects of Pb have been done on humans where it has been

shown that concentrations $<2\text{ }\mu\text{g/dL}$ can cause behavioral problems and adverse health effects in children (Lanphear et al. 2000; Canfield et al. 2003). Using the 97.5th percentile of the national average Pb concentration in children, the Centers for Disease Control and Prevention (CDC) set $5\text{ }\mu\text{g/dL}$ of Pb as a level above which medical attention should be sought for a child. Pokras and Kneeland (2009) argued the relevance of applying human Pb data to wildlife and stressed the known similarities in handling of calcium and Pb (both divalent cations) by the highly conserved mechanisms for calcium metabolism, whether in a human, bird, or fish. These authors point out that Pb interferes with calcium homeostasis and impacts a wide spectrum of physiologic functions, concluding that much of what has been learned about sublethal effects of Pb on humans can be extrapolated to other animals. For example, Fullmer (1991) and Edelsein et al. (1984) documented the complex interactions between calcium and Pb absorption in hatchling white leghorn cockerels (*Gallus gallus domesticus*). Additionally, children and young birds absorb calcium and Pb with greater efficiency than adult humans or birds, making them more susceptible to deleterious effects of Pb (Wasserman and Taylor 1968; Mykkanen and Wasserman 1981). While no guidelines exist for bald eagle nestlings, it is noteworthy that 12 of the nestlings we sampled exceeded the CDC's $5\text{ }\mu\text{g/dL}$ threshold for human children, which may weigh 10 times more than a nestling.

Overall, our study provides an example of using avian nestling feather and whole blood samples to document local exposures to Pb. We found concentrations of Pb in bald eagle nestlings declined from 2006–2015 as evidenced from feather samples, with feathers chronicling both short- and long-term Pb exposures. Concentrations of Pb in whole blood samples of bald eagle nestlings, which document short-term exposures, were generally low when compared to thresholds for adult bald eagles and raptors. However, some bald eagle nestlings had Pb concentrations that approached or exceeded adult thresholds for Pb, and the available literature on humans and other wildlife suggest that bald eagle nestlings could experience sublethal effects at much lower levels.

Acknowledgements We thank the many employees and volunteers at the national parks that assisted with logistics and field sampling. We also appreciate the in-kind support of state, federal, tribal, and non-governmental partners. We thank D. Andersen, J. Glase, B. Lafrancois, C. Morris, D. VanderMeulen, and 2 anonymous reviewers for their technical review of earlier drafts of the manuscript. Use of trade names does not imply endorsement by the U.S. Government or the University of Minnesota.

Funding Major funding was provided by the U.S. National Park Service, Great Lakes Inventory and Monitoring Network, the National Park Service Biological Resources Division, and the Minnesota Pollution Control Agency. The Wisconsin Department of Natural

Resources and Ramsey County (MN) Parks and Recreation provided in-kind support for bald eagle occupancy and productivity flights. JEB was supported as a Research Fellow during data analysis and manuscript preparation at the U.S. Geological Survey, Minnesota Cooperative Fish and Wildlife Research Unit at the University of Minnesota.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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