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Bald eagle mercury exposure varies with region and site elevation in New York, USA

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Abstract

Freshwater fish in several regions of New York State (NYS) are known to contain concentrations of mercury (Hg) associated with negative health effects in wildlife and humans. We collected blood and breast feathers from bald eagle (*Haliaeetus leucocephalus*) nestlings throughout NYS, with an emphasis on the Catskill region to determine their exposure to Hg. We assessed whether habitat type (lake or river), region (Delaware–Catskill region vs. rest of NY) or sample site elevation influenced Hg concentrations in bald eagle breast feathers using ANCOVA. The model was significant and accounted for 41% of the variability in \log_{10} breast feather Hg concentrations. Mercury concentrations in nestling breast feathers were significantly greater in the Delaware–Catskill Region (geometric mean: 14.5 $\mu\text{g/g dw}$) than in the rest of NY (7.4 $\mu\text{g/g dw}$), and greater at nests located at higher elevations. Habitat type (river vs. lake) did not have a significant influence on breast feather Hg concentrations. Geometric mean blood Hg concentrations were significantly greater in Catskill nestlings (0.78 $\mu\text{g/g ww}$) than in those from the rest of NY (0.32 $\mu\text{g/g}$). Mercury concentrations in nestling breast feathers and especially blood samples from the Delaware–Catskill region were generally greater than those reported for most populations sampled elsewhere, including areas associated with significant Hg pollution problems. Bald eagles can serve as valuable Hg bioindicators in aquatic ecosystems of NYS, particularly given their broad statewide distribution and their tendency to nest across all major watersheds and different habitat types.

Keywords Mercury · Hg · *Haliaeetus leucocephalus* · Elevation · Catskill

Introduction

Mercury (Hg) pollution is broadly present in aquatic and terrestrial ecosystems throughout the globe. While a portion of Hg pollution enters ecosystems from natural sources (e.g., volcano emissions, natural Hg deposits) or direct inputs (e.g., chlor-alkali facilities and landfills), the majority originates from anthropogenic sources of air pollution (i.e.,

coal-fired power plants, gold mining, incinerators) (Fitzgerald et al. 1998; Driscoll et al. 2013; Pacyna et al. 2016; Kocman et al. 2017; Streets et al. 2017). Once deposited, sulfur-reducing bacteria and other microbes convert inorganic Hg to its toxic organic form, methylmercury (MeHg) (Gilmour et al. 2013), which readily accumulates in consumers and biomagnifies in both aquatic and terrestrial foodwebs (Atwell et al. 1998; Henny et al. 2003; Cristol et al. 2008; Rimmer et al. 2010; Townsend 2011). In aquatic systems, top predators such as piscivorous birds often have concentrations of MeHg in their tissues ≥ 1 million times higher than concentrations found in water (Watras et al. 1998; Wiener et al. 2003). As a result, piscivorous birds are

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commonly sampled to monitor spatial and temporal patterns of Hg contamination in the environment and to assess Hg risks to organisms (Jackson et al. 2016; Scheuhammer et al. 2016; Evers 2018).

Mercury contamination has been well-documented in fish and wildlife across North America (Evers et al. 1998, 2011; Kamman et al. 2005; Monson et al. 2011; Jackson et al. 2016). Numerous studies have documented adverse impacts of MeHg exposure such as reduced reproductive success, behavioral changes, and neurological problems in a wide variety of bird species (Burgess 2005; Scheuhammer et al. 2007; Ackerman et al. 2016; Whitney and Cristol 2017; Evers 2018). When the adverse impacts of Hg are considered in concert with other stressors (i.e., weather, other contaminants, disturbance, nutrient deficiencies, disease, parasites), it has the potential to have long-term consequences for the stability of wildlife populations (Rimmer et al. 2005; Hallinger and Cristol 2011; Stern et al. 2012; Evers et al. 2014).

Due in part to west to east prevailing wind patterns that facilitate short- and long-distance transport of airborne pollutants, Hg contamination is especially prevalent in northeastern North America (Evers and Clair 2005; Keeler et al. 2005; Driscoll et al. 2007; Evers et al. 2007; Scheuhammer et al. 2016). New York State has long been central to air pollution research and regulatory responses in the United States due in part to interrelated issues of particulate pollution (i.e., SO_x, NO_x), acid rain and Hg pollution, which are often pronounced in high elevation regions of the state such as the Adirondack and Catskill Mountain regions (Driscoll et al. 2003). In a statewide assessment of Hg in sportfish (largemouth bass *Micropterus salmoides*; smallmouth bass *Micropterus dolomieu*; walleye *Sander vitreus* and yellow perch *Perca flavescens*), the New York State Department of Environmental Conservation (NYSDEC) found that Hg concentrations were notably higher in fish from lakes in the Catskill and Adirondack Forest Preserves compared to other regions of the state (Simonin et al. 2008; NYSDOH 2018). These findings were expected because Hg deposition rates are greater in these areas than surrounding regions due in part to their higher elevation and because lake characteristics in these areas (i.e., low productivity, low pH, water level fluctuations) are known to further enhance Hg methylation (Sauer et al. [this issue](#); Miller et al. 2005; Vanarsdale et al. 2005; Driscoll et al. 2007; Yu et al. 2013; Drenner et al. 2013).

Geographic areas with notably elevated concentrations of Hg in biota have been referred to as biological Hg hotspots (Evers et al. 2007). In a regional assessment of Hg in freshwater food webs in the northeastern U.S. and southeastern Canada based upon common loon (*Gavia immer*) and yellow perch bioindicators, Evers et al. (2007) identified the Adirondack Mountains as one of five biological Hg

hotspots, while the Catskill Mountains were identified as one of nine “areas of concern” (these areas did not meet rigorous sample size criteria for hotspot designation, but exceeded specific adverse effect Hg thresholds in two or more secondary data layers). While the common loon served as the primary avian piscivore Hg bioindicator in that study and others (Schoch et al. 2014a; Yang et al. 2019), limitations of the loon’s breeding range to lakes in northern NYS preclude similar use in river systems or in other regions of specific interest, such as the Catskill Mountains. Here, we evaluate Hg exposure in NYS bald eagles (*Haliaeetus leucocephalus*), which nest on lakes and rivers throughout the state and whose exposure to Hg has not been comprehensively evaluated.

Bald eagles are among the most high profile and well-established contaminant bioindicators in North America (Colborn 1991; Bowerman et al. 2002; Elliott and Harris 2002; Golden and Rattner 2003). Numerous natural history traits such as a long lifespan, high trophic status, high fidelity to nesting areas, and diverse nesting habits favor the use of bald eagles in environmental contaminants monitoring (Stalmaster 1987; Palmer et al. 1988; Bowerman et al. 1995; Buehler 2000; Elliott and Harris 2002). While several tissues have been collected from adult bald eagles for use in toxicological assessments (i.e., blood, feather, egg, organs) (Wiemeyer et al. 1989, 1993; Weech et al. 2003; Cristol et al. 2012; DeSorbo et al. 2018), blood and feathers collected from nestlings are most commonly sampled because they can be acquired efficiently and non-lethally during the breeding season (Bowerman et al. 2002; Dykstra et al. 2005). Mercury concentrations in nestling blood and feathers are highly correlated (Welch 1994; Weech et al. 2006; DeSorbo et al. 2018; Kramar et al. 2019) and represent a surrogate for dietary Hg exposure in adults (Wood et al. 1996; Weech et al. 2006), with blood reflecting recent exposure (i.e., hours to days; Bearhop et al. 2000; Fournier et al. 2002; Kenow et al. 2007b; Condon and Cristol 2009) and nestling breast feathers reflecting body reserves and exposure over the 1–3 week period in which feathers were grown prior to sampling (Furness et al. 1986; Ackerman et al. 2011).

While bald eagles have been used extensively to monitor Hg exposure and risk to breeding populations in nearby regions of the Upper Midwest, the Great Lakes and parts of New England (Bowerman et al. 2002; Dykstra et al. 2010, 2019; Pittman et al. 2011; DeSorbo et al. 2018), only one published study to our knowledge has assessed Hg exposure in resident bald eagles in NYS (Wiemeyer et al. 1984; 3 eggs from one nesting territory, 1971, 1977–78). The dramatic recovery of the bald eagle population in NYS since its near extirpation (1 pair in 1970; NYSDEC 2016) is such that the current distribution of the species now enables its use to monitor spatial and temporal patterns of

contaminants in the environment (Fig. S1). Since bald eagles nest in association with multiple habitat types, (i.e., freshwater lakes and rivers, estuarine, marine), broad scale sampling can enable comparisons of contaminants across different watersheds and habitat types in New York. Previous studies have detected differences in tissue Hg concentrations of bald eagle nestlings raised in different habitat types and watersheds (Welch 1994; Evers et al. 2005; DeSorbo 2007; DeSorbo et al. 2009, 2018).

In this study, we characterize Hg exposure in nestling bald eagles throughout interior NYS, with a special emphasis on the Catskill Mountain region due to increased concerns for Hg risks to piscivores in this region. We assessed the influence of region, habitat type (lake versus river) and nest site elevation on Hg exposure in NYS bald eagles.

Methods

Nestling bald eagle handling and tissue sampling

In conjunction with annual nest-checks and eaglet banding, we visited 40 bald eagle nesting territories throughout interior NYS to collect blood and breast feather samples from bald eagle nestlings to be analyzed for Hg (Fig. 1). All nestling samples analyzed in this study were collected in 2006 except one sample collected in 2004. Reconnaissance surveys via fixed wing aircraft or helicopter guided the selection of nest sites containing young approximately 6 weeks of age, the optimal age for handling. Nest success, eaglet age and logistical factors (i.e., landowner permission, tree safety) influenced which nest sites could be sampled. Trees were climbed with spike and lanyard arborist techniques. Nestlings were lowered to the ground and banded with uniquely coded U.S. Geological Survey (USGS) bird bands and uniquely coded anodized blue leg bands (Acraft Sign and Nameplate Co., Edmonton, Canada). Blood and feather samples were generally collected from all nestlings present using methods described elsewhere (DeSorbo et al. 2018). Bald eagle banding and sampling was conducted under the authorization of state and federal permits held by staff of the New York Department of Environmental Conservation, Endangered Species Unit.

Habitat type, region and elevation influences on Hg exposure in bald eagle nestlings

To evaluate the potential influence of habitat type on Hg exposure in bald eagle nestlings, we categorized nest sites visited for sampling into two habitat type categories: lakes (includes reservoirs) and rivers. To evaluate geographic

patterns in Hg exposure, we overlaid HUC-4 watershed boundaries (USGS 2019) and United States Environmental Protection Agency (USEPA) ecoregions (USEPA 2013) over sampling locations in ArcMap 10.6.1 (ESRI 2018) to provide a basis for grouping sample sites for statistical analyses (Fig. 1). Sample sites falling within the Catskill Park, the Catskill High Peaks and Transition region, and the Low Poconos ecoregions, as delineated in the EPA ecoregions coverage (USEPA 2013) were designated as the Delaware–Catskill region. One nest site (NY #29) located 6.8 km north of the Catskill transition ecoregion boundary (Fig. 1) was included in the Delaware–Catskill region rather than the Hudson River watershed where it was physically located because we presumed that the foraging habitat (i.e., mostly lakes) and dietary habits of eagles associated with this nest would be more similar to nests in the nearby Delaware–Catskill region than the other nests sampled in the Hudson River watershed, which were >40 km away and predominantly associated with the Hudson River corridor. Lastly, since factors associated with elevation relate to Hg concentrations in biota sampled in New York (Townsend et al. 2014), we estimated elevation (m above sea level) using the USGS 3DEP GIS coverage (USGS 2017) associated with bald eagle nest sites, and evaluated its influence on bald eagle Hg exposure.

Laboratory analyses

All blood and feather samples collected in this study were analyzed for Total Hg (THg) at the Savannah River Ecology Lab (SREL) at the University of Georgia (Aiken, SC). All laboratory analyses included in this study met United States Environmental Protection Agency (USEPA) quality assurance standards (USEPA 2007a).

Blood

Blood samples contained clots and therefore underwent lyophilization and homogenization prior to analyses. Frozen blood samples were dried to a constant weight utilizing a LabConco FreeZone 4.5 system. Samples were then homogenized to powder consistency with a PTFE pestle grinder. Grinders were cleaned with 0.3 M ultra-high purity nitric acid and ASTM Type 1 water between use. Moisture content was not measured, but dry weight results were converted to wet weight using: $\text{blood Hg } (\mu\text{g/g, ww}) = \text{blood Hg } \text{dw} * (100 - 77\% \text{ moisture}) / 100$, assuming a consistent blood moisture content of 77% (R. Taylor, Trace Element Research Laboratory, College Station, Texas; mean \pm SD: 77.4% \pm 3.7; min-max: 60.5.8–86.2; median 77.4%; $n = 430$; values > 2 SDs from the mean were excluded).

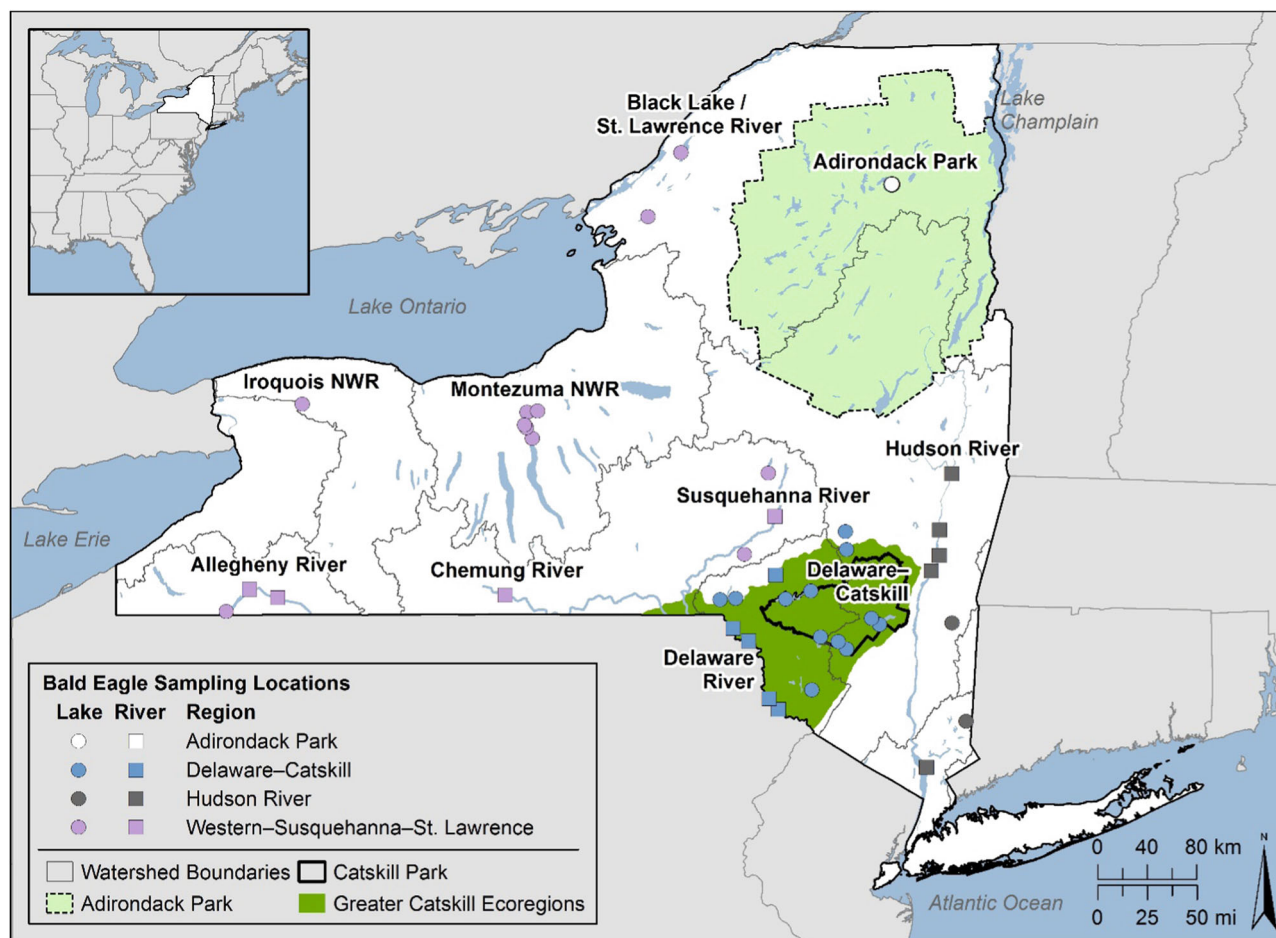


Fig. 1 Location and habitat type associated with sites where tissue samples (blood, feather, or both) were collected from bald eagle nestlings, relative to watershed boundaries, park boundaries and broad geographic regions within New York State in 2004 ($n = 1$) and 2006 ($n = 39$)

Feathers

Individual breast feather samples were washed with a dilute, mercury-free detergent, rinsed with ASTM Type 1 water, and lyophilized to constant weight utilizing a LabConco FreeZone 4.5 system.

Analysis

Samples were analyzed for THg content by combustion-amalgamation-cold-vapor atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA) according to U.S. Environmental Protection Agency (EPA) method 7473 (USEPA 2007b). For quality assurance, each group of 10 to 15 samples included a blank and standard reference material (SRM; TORT-2 lobster hepatopancreas [National Research Council of Canada (NRC), Ottawa, ON] or DORM-2 (dogfish muscle; NRC). Method detection limits (MDL; threefold the standard deviation of procedural blanks) for the samples depended on sample mass and were calculated separately for each observation based on the

mass of sample analyzed. MDLs ranged from 5.31 to 25.43 ng/g dry mass. All the sample concentrations exceeded the detection limits. Mean percent recoveries of THg for the SRMs were $102\% \pm 8\%$ (DORM-2; $n = 16$) for breast feathers, and $96\% \pm 6\%$ (TORT-2; $n = 3$) and $99\% \pm 6\%$ (DORM-2; $n = 2$) for blood. SRMs utilized during analyses encompassed the range of Hg concentrations encountered for each sample set. Matrix matched SRM were unavailable during the time frame of the analyses.

Statistical analyses

Mercury concentrations in blood and breast feathers were averaged at nesting territories for within-year siblings. No nesting territories were sampled in multiple years. We inspected distributions and quantile plots to assess data normality. We transformed blood Hg data using a $\log_{10}(\text{blood Hg} + 1)$ transformation, and we transformed breast feather Hg concentrations using a $\log_{10}(\text{feather Hg})$ transformation to improve the normality and homoscedasticity of the Hg data. We used a Bartlett's test to test for equal variances and then

compared mean Hg concentrations using a t-test for pairs of means, and one-way ANOVA for >2 means. We used a Tukey's HSD test to evaluate pairwise differences among means. We used $|Studentized\ residuals| > 3$ to identify and remove outliers as described in SYSTAT (2009; p.II-10) and Field et al. (2012; p. 269, 292). These references note that large Studentized residuals (>3 in absolute magnitude) indicate outlier values and such values can indicate possible data problems (SYSTAT 2009). Analyses were conducted with and without outliers, which are shown in figures.

Influence of habitat type, elevation and region on Hg concentrations in nestling bald eagles

Given our low and uneven sampling intensity outside of the Delaware–Catskill region, and knowledge that fish Hg was high in this region and in the Adirondacks, evaluations were necessary to determine if we could pool data from different subregions in New York to enable more powerful statistical analyses. We therefore combined Hg data collected in western NY, the Susquehanna River watershed and the St. Lawrence region into one group (Western–Susquehanna–St. L; $n = 14$; Fig. 1), and then compared it to Hg data collected in two other areas, the Adirondacks ($n = 1$) and the Hudson River watershed ($n = 6$), using ANOVA. This grouping resulted in sufficient sample sizes to enable an ANCOVA analysis to evaluate the influence of location within or outside of the Delaware–Catskill region (region), habitat type (lake, river), and sample site elevation on $\log_{10}(\text{feather Hg})$ using ANCOVA. Prior to the ANCOVA, we confirmed that nest site elevation did not confound our regional comparison by analyzing the relationship between breast feather Hg concentrations and elevation within each region. Since the number of blood samples collected in this study was limited, ANCOVA of blood Hg data was not feasible. Lastly, we evaluated the association between mean territory nestling blood Hg concentrations and mean territory nestling feather Hg concentrations using least squares regression.

Statistical analyses were conducted using SYSTAT 13 (SYSTAT Software Inc., San Jose, CA). Geometric means and asymmetric standard deviations are presented in text, tables and figures. Arithmetic summaries of data from this study and other information are presented in the supplemental materials. Results of statistical tests were considered significant at $\alpha = 0.05$.

Results

Hg concentrations in nestling bald eagle tissues

We collected blood ($n = 19$) and breast feather ($n = 44$) samples from nestling bald eagles in 40 nesting territories

within watershed-defined subregions of New York (Fig. 1, Table S1). Mercury was detected in all tissues analyzed. Mercury concentrations in individual nestlings ranged from 0.08–1.44 $\mu\text{g/g}$ ww in blood (overall geometric mean: 0.65 $\mu\text{g/g}$ ww), and 1.2–27.1 $\mu\text{g/g}$ dw in breast feathers (geometric mean: 14.5 $\mu\text{g/g}$ (Fig. 2).

Influence of habitat type, elevation and region on Hg concentrations in nestling bald eagles

Sample sizes in each habitat type and subregion were limited, especially for blood samples. An analysis comparing mean $\log_{10}(\text{feather Hg})$ concentrations among three regions outside the Delaware–Catskill region (Western–Susquehanna–St. L, the Adirondacks and the Hudson River watershed; Fig. 1) indicated there were no significant differences in Hg exposure in nestlings sampled in these three areas ($p = 0.24$, $R^2 = 0.148$, $F_{2,18} = 1.566$). Exclusion of one identified outlier (NY#122 in the Hudson River watershed; Studentized residual: -3.666) did not change the significance of this test ($p = 0.148$, $R^2 = 0.201$, $F_{2,17} = 2.139$). We thus combined breast feather Hg data from these three regions into a single group comprised of samples collected outside the Delaware–Catskill region (rest of NY hereafter). Before evaluating the influence of location within or outside of the Delaware–Catskill region (region), habitat type (lake, river), and sample site elevation on $\log_{10}(\text{feather Hg})$ using ANCOVA, we confirmed elevation did not confound region by evaluating the relationship between $\log_{10}(\text{feather Hg})$ and elevation within each of the two regions. $\log_{10}(\text{feather Hg})$ was not significantly related to elevation within the Delaware–Catskill region ($p = 0.353$, $R^2 = 0.058$, $F_{1,15} = 0.920$; range: 178–439 m); however the relationship between these two variables was significant within the rest of NY group ($p = 0.0269$, $R^2 = 0.232$, $F_{1,19} = 5.750$; range: 0–497 m), which had a wider range in elevations. The relationship between feather Hg and elevation remained highly significant after we removed one outlier (NY#122; Studentized residual: -4.349 ; $p = 0.00434$, $R^2 = 0.232$, $F_{1,18} = 10.637$).

Given the significant association between Hg and elevation within the rest of NY group and overall, we concluded elevation did not confound region and proceeded with an ANCOVA. The ANCOVA model was significant and accounted for 41% of the variability in $\log_{10}(\text{feather Hg})$ (Table 1). Mercury concentrations in nestling breast feathers were greater in the Delaware–Catskill region than in the rest of NY (Fig. 2, Table 2, Fig. S1, Table S2), but were also greater at nests located at higher elevations (Fig. 3). Habitat type did not have a significant influence on feather Hg. The ANCOVA model remained significant after the removal of a single outlier (NY#122; Studentized residual: -4.276 ; Table S3). Similar to Hg concentrations in breast feathers, geometric mean blood Hg concentrations

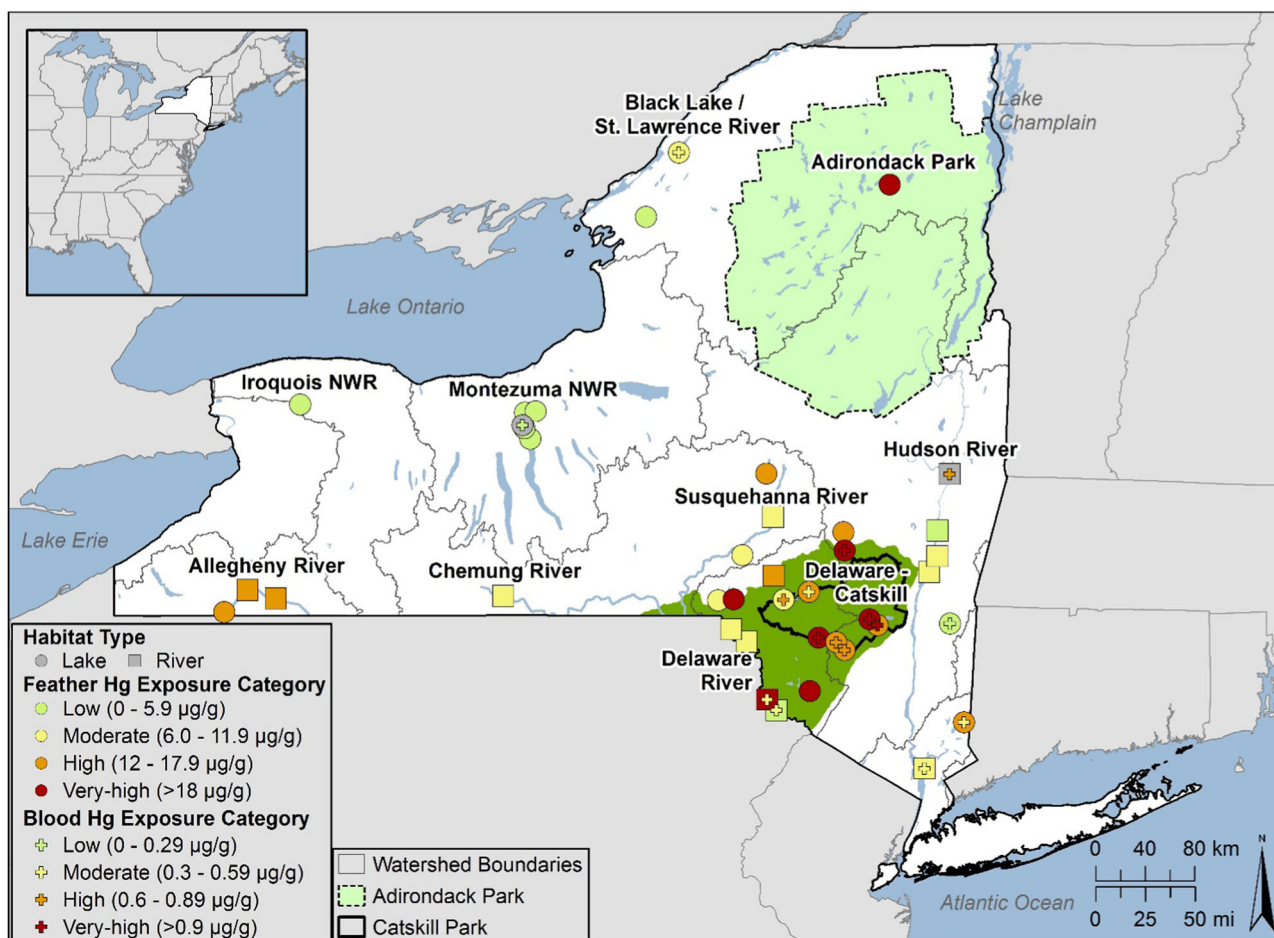


Fig. 2 Mercury concentrations in blood and breast feathers of bald eagle nestlings sampled in the Delaware–Catskill region and the rest of New York (rest of NY) in 2004 ($n = 1$) and 2006 ($n = 39$)

Table 1 Analysis of Covariance (ANCOVA) results of the impact of region, habitat type and sample site elevation on nestling bald eagle breast feather Hg concentrations (\log_{10} [feather Hg]) in New York State, USA^{a,b}

Source	<i>df</i>	Mean squares	<i>F</i>	<i>P</i>
Region	1	0.349	6.0771	0.0136*
Habitat type	1	0.0169	0.329	0.5699
Elevation	1	0.409	7.926	0.0081*
Error	34	0.0516	–	–

^a $R^2 = 0.41$

^bBefore ANCOVA was conducted, regression equations were determined to exhibit homogenous slopes

*Indicates significant test result ($p < 0.05$)

were significantly greater in Delaware–Catskill nestlings than in those from the rest of New York ($n = 16$, $t_{14} = -3.44$, $p = 0.004$) (Tables 2, S2, S4, Fig. S2). Low sample sizes precluded statistical comparisons of Hg concentrations in nestling blood samples collected in lake versus river habitat types (Tables S5, S6).

Relationship between Hg concentrations in nestling blood and breast feathers

We assessed the association between Hg concentrations in nestling breast feather and blood samples collected from the same nesting territory. Mean territory breast feather Hg concentrations were significantly related to mean territory blood Hg concentrations (Fig. 4). A single low Hg outlier exerted significant leverage on the regression. Nevertheless, the blood-feather Hg relationship remained significant after excluding this outlier.

Discussion

This study provides the first comprehensive assessment of Hg exposure in the NYS bald eagle population. Our findings revealed that Hg concentrations in nestling bald eagle tissues (blood, feather) nearly spanned the range of Hg exposure observed in populations elsewhere (Pittman et al.

Table 2 Geometric mean, lower and upper asymmetric standard deviations, range and sample sizes of blood and breast feather Hg concentrations in bald eagle nestlings sampled in two different regions of New York, USA, and arithmetic mean and standard deviation of sample site elevation (m above sea level)

Region	Blood Hg, $\mu\text{g/g}$ ww				Breast feather Hg, $\mu\text{g/g}$ dw				Elevation (m)		
	<i>n</i>	GeoMean	Lower SD	Upper SD	<i>n</i>	GeoMean	Lower SD	Upper SD	<i>n</i>	Mean	SD
Delaware-Catskill	10	0.78 (0.33–1.41)	0.49	1.13	17	14.5 (5.3–25.7)	9.5	22.1	17	322 (178–440)	95
Rest of NY	6	0.32 (0.09–0.63)	0.13	0.54	21	7.4 (1.2–21.1)	3.9	14.3	23	198 (0–497)	167
Overall	16	0.59	0.27	1.0	38	10.0	5.2	19.2	40	251	153

Min–max values in parentheses

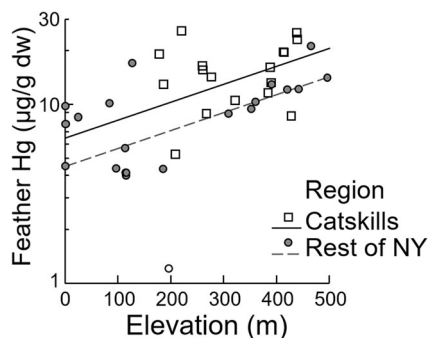


Fig. 3 ANCOVA regression plot of the relationship between elevation and breast feather Hg concentrations in bald eagle nestlings sampled in the Delaware–Catskill region (Catskills) and areas outside of the Catskill region (rest of NY) in 2004 ($n = 1$) and 2006 ($n = 39$). The ANCOVA Model was significant both including (Table 1) and excluding the outlier (open circle; Table S2)

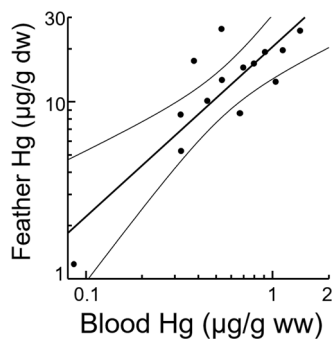


Fig. 4 Linear regression plot of \log_{10} (blood Hg + 1) and \log_{10} (breast feather Hg) concentrations in bald eagle nestlings in New York State ($p = 0.003$, $R^2 = 0.54$, $n = 14$; $\log_{10}[\text{breast feather Hg}] = 2.6595 \cdot \log_{10}[\text{blood Hg} + 1] + 0.5012$). The blood-Hg relationship remained significant after exclusion of the low Hg outlier: ($p = 0.027$, $R^2 = 0.37$, $n = 13$; $\log_{10}[\text{breast feather Hg}] = 1.4722 \cdot \log_{10}[\text{blood Hg} + 1] + 0.8089$). Curved lines show 95% confidence interval

2011, DeSorbo et al. 2018), with some individuals exhibiting notably elevated tissue Hg concentrations. Our analyses indicated that Hg concentrations in breast feathers collected from bald eagle nestlings were influenced by their location relative to the Delaware–Catskill Region and nest site elevation, but not by habitat type associated with the nest.

Many of the factors affecting geographic patterns of MeHg availability in freshwater and terrestrial ecosystems have been well-described (Driscoll et al. 2003, 2007; Evers et al. 2007; Munthe et al. 2007; Simonin et al. 2008; Eagles-Smith et al. 2016). In general, freshwater aquatic systems with elevated MeHg availability often exhibit some combination of characteristics including low productivity, abundant wetlands, regular wetting and drying cycles, and specific water chemistry parameters that promote Hg methylation and biomagnification (i.e., SO_4 , pH, acid neutralizing capacity [ANC], dissolved organic carbon) (Abernathy and Cumbie 1977; Grigal 2003; Kramar et al. 2005; Chen et al. 2005; Chumchal et al. 2008; Simonin et al. 2008; Eagles-Smith et al. 2016; Broadley et al. 2019). These and other factors promote a high degree of landscape sensitivity to Hg inputs, creating the potential for development of a “biological Hg hotspot”—an area with notably elevated concentrations of Hg in biota relative to the surrounding landscape (Evers et al. 2007).

The finding in our study that Hg concentrations were elevated in nestling bald eagle tissues sampled in the Delaware–Catskill region is consistent with previous indications that the Catskill Park region is an area of concern for Hg contamination in northeastern North America (Miller et al. 2005; Evers et al. 2007). This region is subject to higher rates of Hg deposition compared to surrounding areas as a result of its higher elevation and location downwind from regional and distant Hg pollution sources to the south and west (Miller et al. 2005; Vanarsdale et al. 2005; Ye et al. 2019). Unique water chemistry parameters in this region further enhance Hg bioavailability. Simonin et al. (2008) noted that the pH, ANC and calcium concentrations were 10 times lower in lakes within the Adirondack and Catskill Parks compared to lakes outside these areas. The Delaware–Catskill region also contains numerous reservoirs, including several large impoundments that are part of the New York City water supply. Changes in Hg bioavailability have been linked to a number of physical, chemical and biological changes associated with the flooding of terrestrial habitats and regular wetting and drying cycles in the littoral zone (Verta et al. 1986; Schetagne and Verdon 1999; Snodgrass et al. 2000). Of the 10

lakes (12 nesting territories) we sampled in the Delaware–Catskill region, 90% were impounded, compared to 53% of lakes in the rest of NY group (7 of 13). Previous studies have suggested that water level changes may contribute to elevated Hg levels in fish observed in the Catskill Park and elsewhere in NYS (NYSDEC 2008; Simonin et al. 2008).

Elevated Hg concentrations have been noted in other biota in the Delaware–Catskill region. Townsend et al. (2014) found that Hg concentrations increased in forest floor horizons, red-backed salamanders *Plethodon cinereus* and *Catharus* thrushes along an increasing elevational gradient in the Catskill Forest Preserve, and raised concerns about the long-term release of high elevation Hg into lower elevation watersheds. Along with the Adirondack region, the Catskill Mountain region was highlighted as an area of concern in a statewide assessment of Hg concentrations in predatory fish (Simonin et al. 2008). Analyses of several contaminants in fish from the New York City reservoir system determined Hg was the primary contaminant of concern in fish tissue, with fish exceeding the USEPA piscivorous wildlife criterion for Hg in high trophic level fish (346 ng/g; USEPA 1997) in 50% of the samples from 13 fish species at all 16 reservoirs sampled (NYSDEC 2005). Mercury contamination was prominent in 10 reservoirs in particular, including four east of the Hudson River and all six reservoirs west of the Hudson River within the Delaware–Catskill region (all six were sampled in the present study; Table S1) (NYSDEC 2005, Simonin et al. 2008; see also NYSDEC 2006a, b). The New York State Department of Health (NYSDOH) subsequently issued fish consumption advisories at 11 additional NYS reservoirs, bringing the current total to 14 of 19 reservoirs in NYS (NYSDOH 2018). No comparison data exists to our knowledge on Hg exposure in other piscivorous birds in the Delaware–Catskill region.

Positive relationships between Hg and elevation have been previously noted in biotic and abiotic studies (Lawson et al. 2003; Miller et al. 2005; Townsend et al. 2013, 2014; Yu et al. 2013). Meteorological conditions such as increased cloud and fog cover and higher precipitation rates are important to increasing total Hg deposition at elevation (Vanarsdale et al. 2005; Driscoll et al. 2007; Townsend et al. 2014), while habitat characteristics at elevation such as increased coniferous forest cover further facilitate atmospheric Hg exchange and enhance MeHg bioavailability (Yu et al. 2013; Drenner et al. 2013; Townsend et al. 2014). High elevation habitats also receive greater inputs of acidic deposition and sulfates, which further enhance Hg methylation (Driscoll et al. 2003; Burgess and Meyer 2008; Riva-Murray et al. 2011; Schoch et al. 2014b). Although no other studies have assessed the relationship between Hg and elevation in bald eagles, we suspect this association will be

unique to mountainous regions such as those in NYS, where bald eagles nest across a wide elevational gradient (Table 2) and also experience high Hg inputs to areas with site characteristics facilitating Hg methylation.

In our study, habitat type (lake vs. river) did not have a significant influence on Hg concentrations in nestling feathers. This finding is contrary to that for bald eagles studied in Maine, where Hg exposure was greater in nestlings raised near lakes rather than rivers (Welch 1994; Evers et al. 2005; DeSorbo et al. 2018). Higher Hg exposure at lake versus river sample sites has also been documented in other piscivores such as belted kingfishers (*Megaceryle alcyon*) and numerous freshwater fish (Kamman et al. 2005; but see Pennuto et al. 2005). Higher flushing rates at rivers and related dilution effects on Hg are considered to be among the most important factors driving differences in MeHg bioavailability between lake and river habitats (Fimreite 1974; Evers et al. 2005). Other factors that promote MeHg production such as low pH (i.e., <6.3), high dissolved organic carbon, and fluctuating water levels are generally more characteristic of freshwater lakes than rivers (Meyer et al. 1995; Chen et al. 2005; Burgess and Hobson 2006). Lacking differences in feather Hg concentrations between nestlings from lake and river habitat types in our study may be related to low sample sizes of habitat types represented within individual watersheds sampled. In our study, Hg concentrations appeared higher at lake vs. river sites within some subregions (i.e., Delaware–Catskill region; Tables S5, S6), but not in others, a pattern also observed in major watersheds across Maine (DeSorbo 2007; DeSorbo et al. 2009). Similarities in bald eagle Hg exposure between these habitat types may also reflect variation in the eagles' diets, particularly when abundant food is available in a different habitat type nearby. While inland bald eagles predominantly prey upon fish local to the nest when available (Todd et al. 1982; Thompson et al. 2005), bald eagles are not strictly piscivorous and dietary emphasis on non-fish prey (i.e., herbivorous mammals and birds) and some anadromous fish can lower Hg exposure. As such, prey remains collected at the nest site identified as a low Hg outlier in our analyses (NY#122; P. Nye, unpublished data) indicated heavy dietary emphasis on mallards (*Anas platyrhynchos*), which are often low in Hg (Evers et al. 2005; Hall et al. 2009).

Mercury exposure outside the Delaware–Catskill region

Our ability to assess Hg exposure patterns in nestling bald eagles sampled in specific subregions outside the Delaware–Catskill region is limited due to small sample sizes relative to the area we sampled throughout NYS. Past statewide assessments of Hg in fish indicated that in

addition to the Catskill Forest Preserve, Hg concentrations were also elevated in the Adirondack region (NYSDEC 2008; Simonin et al. 2008). In our study, the breast feather sample with the highest Hg concentration in the rest of NY region originated from the Adirondack Park (21.1 µg/g). Evidence of elevated Hg exposure in the Adirondack region in fish, songbirds and common loons has been demonstrated in other studies (Evers et al. 2007; Simonin et al. 2008; Yu et al. 2011; Schoch et al. 2014a; Burns and Riva-Murray 2018; Driscoll et al. 2019, Sauer et al. [this issue](#)). Further sampling is needed to assess Hg risk to bald eagles residing in the Adirondack region. Sampling in our study also is suggestive that bald eagles in the Allegheny River watershed in the southwestern corner of the state may be exposed to elevated Hg levels (range: 12.1–14.0 µg/g, $n = 3$), a finding consistent with fish consumption advisories issued in Pennsylvania for the Allegheny River and reservoir due to mercury and PCB contamination (PADEP (2018). There are no fish consumption advisories for the Allegheny River and reservoir in NYS (NYSDOH 2018).

Mercury in nestling bald eagles—population comparisons

The geometric mean Hg concentration in bald eagle nestling blood from in the Delaware–Catskill region (0.78 µg/g ww) was greater than that found in most bald eagle populations elsewhere in North America. This includes contaminated sites such as Pinchi Lake, British Columbia (0.57 µg/g ww; associated with a Hg mine; Weech et al. 2006) and Maine lakes, which are notably contaminated with Hg from atmospheric deposition (0.56–0.62 µg/g; (DeSorbo et al. 2009, 2018); see also Evers et al. 2008). Only two bald eagle studies reported higher average blood Hg concentrations than bald eagles sampled in the Delaware–Catskill region: (1) a severely contaminated region in western Oregon (1.2 µg/g, $n = 82$; Wiemeyer et al. 1989), and (2) a subset of nests ($n = 3$) sampled along the South Fork of the Shennandoah River in inland Virginia (0.80 µg/g; Kramar et al. 2019, D. Kramar, personal communication). As reflected by Hg exposure in birds, the South Fork of the Shennandoah River is among the most severely polluted rivers in the country (Brasso and Cristol 2008; Jackson et al. 2011). The mean Hg concentration in blood of bald eagle nestlings throughout inland (primarily central) Virginia was 0.32 µg/g (Kramar et al. 2019), the same mean concentration we found in nestlings from the rest of NY region in the present study.

In contrast to population comparisons of Hg concentrations in blood samples, mean breast feather Hg concentrations in nestlings from the Delaware–Catskill region (14.5 µg/g) were lower than those from Pinchi Lake, BC (18.0 µg/g), lakes in the Penobscot River watershed in

Maine (19.6 µg/g), and 3 nests in the Shennandoah River in Virginia (18.9 µg/g; D. Kramar, personal communication, Kramar et al. 2019). Mercury concentrations in nestling feathers from the Delaware–Catskill region were also lower than those sampled at lakes in Voyageurs National Park in Minnesota prior to water level stabilization in the late 1980s, a period associated with notable Hg contamination (20.0 µg/g; Bowerman et al. 1994). Mercury concentrations in nestling feathers from the Delaware–Catskill region were higher than those found throughout central/interior Virginia (8.4 µg/g; Kramar et al. 2019) and throughout the Great Lakes region during the late 1980s (3.7–8.8 µg/g; Lakes Superior, Michigan, Huron and Erie; interior areas in the upper and northern lower peninsulas of Michigan; Bowerman et al. 1994).

The mean Hg concentration in nestling feathers sampled outside the Delaware–Catskill region in our study (rest of NY; 7.4 µg/g), was higher than that found in several comparison regions, including Kabetogama Lake (1.1 µg/g; Voyageurs N.P., Pittman et al. 2011), Lake Erie (3.7 µg/g; Bowerman et al. 1994), Florida (3.5–4.7 µg/g; Wood et al. 1996), Namakan Lake (5.6 µg/g) and Rainy Lake (6.1 µg/g) in Voyageurs National Park (Pittman et al. 2011), six sites in the Upper Midwestern U.S. (2.69–6.6 µg/g; Dykstra et al. 2010, 2019) and multiple lakes with varying degrees of natural Hg deposits used as references for Pinchi Lake in British Columbia (7.1 µg/g).

Overall, given typical levels of variability associated with Hg concentrations in blood (SDs range from 0.07–0.27 µg/g) and nestling breast feathers (SDs range from 1.5–8.5 µg/g) reported in the literature (see review in DeSorbo et al. 2018), findings in our study suggest that Hg is elevated in bald eagles residing in portions of NYS, particularly the Delaware–Catskill region, the Catskill Park (Table S7), and probably discrete areas outside this region. Mercury may be similarly elevated in other piscivores within the Delaware–Catskill region.

Toxicological risk of Hg to bald eagles in New York state

While numerous studies have found evidence that elevated levels of Hg exposure are linked to a variety of adverse effects in birds (Ackerman et al. 2016; Evers 2018), neither adverse effect thresholds or effect concentrations have been proposed for Hg in bald eagle blood or feathers (see recent discussions in DeSorbo et al. 2018, Dykstra et al. 2019). Of the published studies to date that evaluated the relationship between Hg and reproduction in bald eagle populations contaminated with moderate to high levels of Hg (Frenzel 1984; Anthony et al. 1993; Bowerman et al. 1994; Welch 1994; Weech et al. 2006), none detected evidence that Hg limited reproduction (see also Dykstra et al. 2019 and

Helander et al. 1982). In most of these studies however, high concentrations of other contaminants (i.e., DDE, PCBs) known to negatively affect reproduction in eagles confound assessments of Hg effects. In a post mortem bald eagle study, Rutkiewicz et al. (2011) reported that 14–27% of eagle carcasses collected in the Great Lakes region ($n = 135$; most of them adults) were exposed to Hg at concentrations associated with subclinical neurological damage, a finding similarly reported for bald eagles analyzed across 7 Canadian provinces (Scheuhammer et al. 2008; see also Weech et al. 2003). Comparisons of bald eagle Hg exposure in the present study with those reported elsewhere suggest that a portion of bald eagles in NYS are likely exposed to similar or higher Hg levels to those analyzed in these neurochemical studies (DeSorbo et al. 2008; Pittman et al. 2011; Rutkiewicz et al. 2011; Dykstra et al. 2019).

Since the majority of avian toxicological research investigations emphasize adult and post-fledged juvenile bird age classes to avoid influences of mass dilution and extensive feather growth on circulating blood Hg levels, adverse effect concentrations delineated in these studies have limited application for interpreting of Hg concentrations as measured in the present study. There are however, several studies that detected adverse health effects of Hg in developing nestlings with tissue concentrations in the range of those we found in NYS bald eagle nestlings. Concentrations of 0.66 $\mu\text{g/g}$ Hg in blood were associated with oxidative stress, altered glutathione metabolism and immune suppression in 5-week old common loon chicks dosed with 0.4 $\mu\text{g/g}$ Hg (Kenow et al. 2007a, 2008), while numerous negative effects (neurological, immunological, histological, reduced appetite, growth, activity and willingness to hunt prey) were documented in juvenile great egrets (*Ardea albus*) dosed with 0.5 $\mu\text{g/g}$ Hg (Bouton et al. 1999; Spalding et al. 2000b, a). Past studies indicate concentrations of 0.4 $\mu\text{g/g}$ or 0.5 $\mu\text{g/g}$ Hg are environmentally realistic in some fish species sampled at lakes throughout NYS (NYSDEC 2005, 2006a; Simonin et al. 2009) and these levels are associated with severe negative effects in adult common loons (Burgess and Meyer 2008; Depew et al. 2012). Of nesting territories in which blood samples were collected from nestlings in the present study, 63% ($n = 10$ of 16) exhibited mean blood Hg concentrations $>0.5 \mu\text{g/g}$, and 50% were $>0.66 \mu\text{g/g}$ (all but one of these 10 territories was located in the Delaware–Catskill region). Recently fledged bald eagles exposed to high Hg prey could be particularly vulnerable to adverse physiological and neurological effects of Hg, as growing feathers no longer provide a protective excretory route for Hg during this consequential period in their development (Fournier et al. 2002; Ackerman et al. 2011). Bald eagles may have a lower susceptibility to MeHg toxicity compared to other well-

studied avian bioindicators such as common loons due to enhanced abilities to metabolize MeHg and Se in organs (Norheim and Frøslle 1978; Scheuhammer et al. 2008).

The NYS bald eagle population is growing rapidly and exhibits favorable growth and reproduction measures (NYSDEC 2016). In 2006, the primary sampling year in the present study, productivity and nest success for the statewide population were 1.55 chicks per occupied nest and 75% (percentage of occupied nests successful in hatching ≥ 1 chick) respectively (P. Nye, pers. comm.), well above the generally accepted productivity level of 1.0 young per occupied nest associated with population stability (Wiemeyer et al. 1984). Reproduction parameters for the nests sampled in the Delaware–Catskill region exhibited similarly healthy productivity levels in 2006 (NYSDEC 2006b).

The role of bald eagles in future Hg monitoring in NYS

Avian piscivores are an important component in spatial and temporal contaminant monitoring programs. The common loon, an obligate piscivore, has been central to Hg biomonitoring programs in NYS (Schoch et al. 2014a, Yang et al. 2019). However, limitations of the current breeding range of common loons to northern NYS (Evers et al. 2010) prevent its use for statewide contaminants monitoring. The incorporation of bald eagles into existing biomonitoring efforts would improve current efforts to investigate Hg risk to upper trophic level wildlife across all of NYS.

Although a comprehensive statewide bald eagle survey has not been conducted in New York since 2014 (254 occupied nesting territories; NYSDEC 2016), the number of occupied nesting territories in 2018 has been estimated to be 383 (Nye 2009; P. Nye, unpublished data), with pairs inhabiting all major watersheds and major river and lake systems in the state, including the Great Lakes shorelines (Fig. S3). Bald eagles have been central to ongoing long-term contaminant monitoring programs in nearby regions including the Great Lakes (Bowerman et al. 1994, 2002; Roe 2004; Wierda 2009) and the Upper Midwestern U.S. (Route et al. 2011, 2019; Dykstra et al. 2019). Further investigations in NYS are recommended to better understand the Hg exposure patterns observed in this study and to track changes over time.

Conclusions

Findings presented in this study indicate that bald eagles in portions of NYS are exposed to elevated Hg concentrations, presumably through the consumption of Hg-contaminated prey. Our study demonstrates that there are regional and elevational influences on Hg exposure in bald eagles, with

greater exposure in the Delaware-Catskill region and in nesting territories at higher elevations. Mercury concentrations in tissues of bald eagles in the Delaware-Catskill region are comparable to or higher than those found in populations with significant Hg pollution issues, either from direct inputs or atmospheric deposition. It remains unclear whether or how Hg might be affecting individual bald eagles within the NYS population; however, any potential effects on reproduction would appear to be insufficient in outpacing the strong continuing recovery of the NYS population. Additional nestling sampling is needed to improve geographic assessments of Hg exposure initiated in this study, particularly given known fish Hg issues elsewhere in the state (i.e., Adirondacks, Hudson River; Baldigo et al. 2006; Levinton and Pochron 2008). Future sampling of adult and subadult bald eagles would address a significant datagap (especially in areas of elevated fish Hg) and would also improve our ability to assess Hg risks in these key age classes.

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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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