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Mercury concentrations in bald eagles across an impacted watershed in Maine, USA

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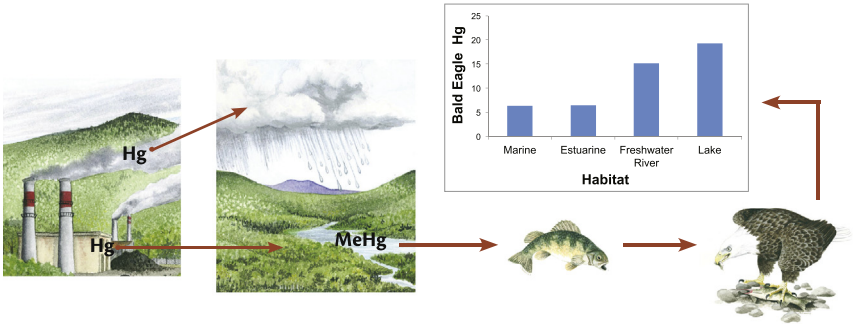
HIGHLIGHTS

- We compared the influence of a chlor-alkali plant and habitat on eagle Hg exposure.
- Hg exposure was higher in the zone impacted by the plant than the reference.
- Eagle Hg differed significantly among lake, river, estuarine and marine habitats.
- Habitat type was more important than the chlor-alkali plant in influencing eagle Hg.
- Eagle Hg exposure was high relative to other populations and effects concentrations.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury (Hg) exposure was evaluated in bald eagles (*Haliaeetus leucocephalus*) in the lower Penobscot River watershed (PRW) in Maine to assess whether Hg discharges from a chlor-alkali plant (HoltraChem) influenced Hg concentrations in nestling tissues. Mean Hg concentrations in nestling blood and breast feathers sampled in marine and estuarine areas potentially contaminated with Hg from HoltraChem (the potential Hg impact zone) were significantly greater than those from reference sites spanning the Maine coast. To place Hg exposure in the potential Hg impact zone into a broader context, Hg exposure in bald eagle nestlings from four habitat types in the PRW was assessed. Mercury concentrations varied significantly across habitat types within the PRW, generally following the pattern: marine = estuarine < freshwater river < lake. While findings suggest that Hg inputs from HoltraChem elevated Hg concentrations in eagles in the potential Hg impact zone, those Hg concentrations were still significantly lower than those of nestlings raised in freshwater river and lake habitats in the PRW and elsewhere in Maine not contaminated by HoltraChem. Breast feathers had 31% higher statistical power to detect Hg differences among habitat types compared to nestling blood, demonstrating their higher value in biomonitoring efforts. Nestling tissue Hg concentrations in the PRW were within the range of reported Hg values for bald eagles, but were generally higher than most population comparisons within habitats. Mercury concentrations in lake-nesting bald eagles in the PRW were impacted primarily by inputs from atmospheric deposition, and Hg exposure in nestlings associated with this habitat type in the PRW often had similar or higher Hg

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exposure than those associated with point sources elsewhere. Mercury concentrations in bald eagle nestlings and a small sample of adults in our study commonly exceeded levels associated with adverse health effects in other bird species.

1. Introduction

The Penobscot River watershed in Maine, USA is the second largest river basin in New England. With an area of 22,196 km^2, the Penobscot River Watershed is the largest in Maine, draining approximately 24% of the state. Waters of the Penobscot originate in northern Maine and extend over 200 km south to Penobscot Bay. In the late 1960s and early 1970s, a chlor-alkali facility in Orrington, Maine, HoltraChem, deposited an estimated 6 to 12 metric tonnes of Hg into the tidally influenced section of the Penobscot River Estuary (Bodaly et al., 2018, 2009a). In 2002, the U.S. District Court, District of Maine, ordered the initiation of Phase I of Penobscot River Mercury Study (PRMS), which entailed measuring Hg concentrations in water, wetlands, sediments and biota (fish, shellfish, invertebrates, birds, and mammals) in the Penobscot River watershed (PRW) and reference sites, and assessing the potential that Hg contamination might pose health risks to biota or humans consuming fish and shellfish. The bulk of this work occurred during 2006–2014. The conclusion of the PRMS was that a 25 km stretch of the lower Penobscot River was significantly contaminated with Hg (Bodaly et al., 2018; Rudd et al., 2018). Sediments were most contaminated with Hg in river reaches extending approximately 6.5 km upstream (to the I-395 bridge in Brewer), and 30 km downstream (to the south end of Verona Island) from HoltraChem (Fig. 1) (Yeager et al., 2018). While sediment sampling also confirmed that additional historic sources of Hg existed further upstream from this zone, patterns of Hg contamination in sediments, invertebrates, fish and some birds supported the hypothesis that HoltraChem was the dominant source of Hg to the lower Penobscot River and upper Penobscot Bay.

Mercury concentrations in invertebrates, fish, and birds sampled within the area contaminated by Hg from HoltraChem were high relative to reference areas (Bodaly et al., 2009a; Kopec et al., 2018a, 2018b; Rudd et al., 2018) and other contaminated sites in the region and elsewhere (Evers et al., 2007; Jackson et al., 2015). Mercury concentrations in tissues of several species consumed by humans, including American lobsters (*Homarus americanus*) and American black ducks (*Anas rubripes*) sampled in the Penobscot River Estuary exceeded the limit established by the Maine Department of Environmental Protection to protect human health (0.20 μg/g ww), prompting closures and health advisories by state regulatory agencies in 2011 (the Department of Inland Fisheries and Wildlife and the Maine Center for Disease Control warned against eating breast muscle from ducks harvested in the Lower Penobscot River) and 2014 (the Maine Department of Marine Resources closed a portion of the river to lobster harvesting) (Kopec et al., 2018a; Rudd et al., 2018; Sullivan and Kopec, 2018). Additionally, Hg

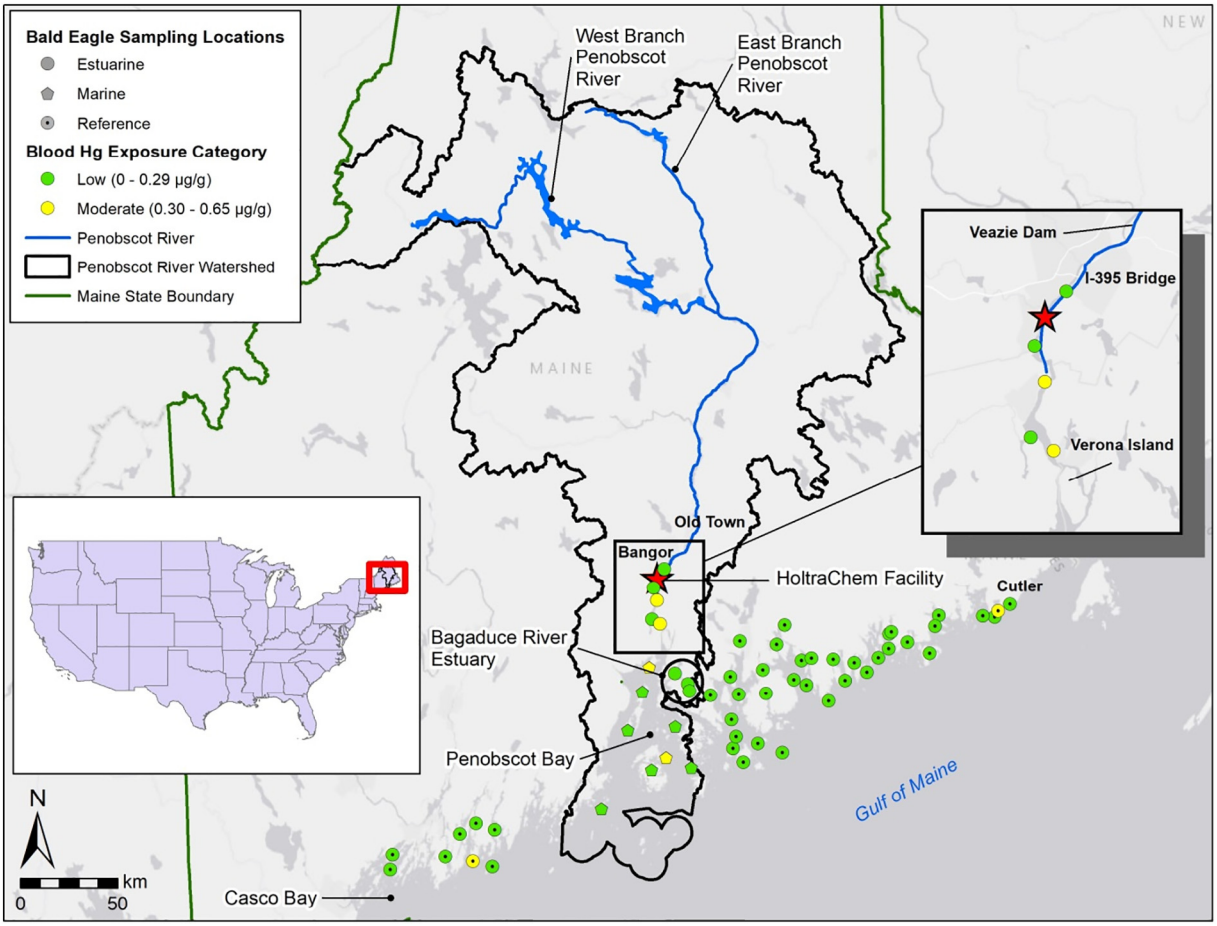

Fig. 1. Bald eagle nest sites sampled in the lower Penobscot River, Penobscot Bay, and marine-based reference sites along the Maine coastline. (online version in color).

concentrations in several fish species sampled in the lower Penobscot River (i.e., tomocod *Microgadus tomocod*, American eel *Anguilla rostrata*, golden shiners *Notemigonus crysoleucas*; Bodaly et al., 2009a, 2009b; Kopec et al., 2018a) exceed wildlife dietary screening benchmarks in prey fish associated with adverse health effects on piscivorous birds (0.1–0.4 μg/g ww; Depew et al., 2012). Mercury concentrations in blood and egg samples collected from multiple bird species in the lower Penobscot River and Bay (i.e., passerines, marsh birds, black guillemot *Cepphus grylle*, double-crested cormorant *Phalacrocorax auritus*; (Bodaly et al., 2009a; Kopec et al., 2018b) exceeded the EC_{20} level (the "effects concentration" associated with a 20% reduction in reproductive success; Van der Hoeven, 1997) for Hg in the blood of adult piscivorous birds (2.0 μg/g ww) and eggs (0.65 μg/g ww) (Evers, 2018).

Mercury (Hg) contamination is prevalent in aquatic habitats around the world (Driscoll et al., 2013). Inputs of Hg into the environment originate from both natural (i.e., volcanoes, bedrock) and anthropogenic sources. Anthropogenic inputs are generally related to either direct discharges of Hg into soils and waterways or atmospheric deposition, generally related to the combustion of fossil fuels and Hg-containing products (Driscoll et al., 2013). Once deposited on the landscape, elemental and inorganic Hg inputs are transformed by sulfur-reducing bacteria into methylmercury (MeHg), a toxic form of Hg that readily bioaccumulates in organisms and biomagnifies up food webs (Atwell et al., 1998; Driscoll et al., 2007; Eagles-Smith et al., 2016). A wide variety of factors influence the rate at which inorganic Hg inputs are transformed to MeHg, including water temperature, pH, salinity, levels of dissolved organic carbon, sulfur, and selenium (Wiener et al., 2003). Since the factors influencing Hg methylation rates vary substantially according to site-specific habitat characteristics (i.e., amount of wetland habitat, extent of water level fluctuations), MeHg production and bioaccumulation in organisms can be notably decoupled from Hg inputs (Eagles-Smith et al., 2016).

Bald eagles (*Haliaeetus leucocephalus*) are one of the most well-established contaminant bioindicator species in North America (Colborn, 1991; Elliott and Harris, 2002). The high trophic level feeding habits, long lifespan, high territory fidelity, and willingness to nest in a wide variety of habitats make them ideal for environmental monitoring of contaminants (Bowerman et al., 2002; Buehler, 2000). While contaminant concentrations are reported in a wide variety of tissue types (i.e., eggs, adult feathers, liver), nestling blood and feather tissues are commonly sought by researchers in environmental monitoring because they can be collected efficiently and non-lethally at specific nest sites in quantities that support robust study designs.

Maine's bald eagle population has been used to monitor environmental contaminants throughout much of its long recovery history. In two nationwide assessments of Hg, organochlorine pesticides and PCBs in bald eagle eggs sampled in 1969–1979 and 1980–1984, Maine's bald eagle population had the highest Hg concentrations of all states sampled (Wiemeyer et al., 1993, 1984). In 1991–1992, Welch (1994) measured Hg concentrations in nestling blood and feathers sampled broadly throughout Maine. Her results did not suggest any notable decreases in Hg in the bald eagle prey base, and also demonstrated that Hg exposure in nestlings increased by habitat type when moving inland from the coast (marine < estuarine < river < lake). Further studies in Maine revealed that the pattern of higher Hg concentrations in bald eagle tissues at lakes relative to rivers was evident within individual watersheds, and that Hg contamination was pervasive throughout much of interior Maine (DeSorbo, 2007; DeSorbo et al., 2009).

The objectives of this study were to determine if Hg inputs from the HoltraChem plant were associated with elevated Hg concentrations in bald eagle nestlings sampled in the lower Penobscot River and Penobscot Bay (the potential Hg impact zone; see Section 3.1), and to put any observed elevation of eagle Hg exposure into context within the larger PRW. Specifically, the following hypotheses were tested: (1) Null Hypothesis (Ho): there is no significant difference in tissue (blood, feather) Hg concentrations between bald eagle nestlings in the potential Hg impact zone versus nestlings from reference areas in Maine; (2) Ho: there is no significant difference in tissue Hg concentrations between bald eagle nestlings in freshwater lake, river, estuarine, and marine habitats within the PRW; and (3) Ho: there is no significant difference in bald eagle nestling blood and breast feathers for use in Hg biomonitoring. Lastly, blood samples were collected from adult bald eagles at lakes to establish a baseline blood Hg exposure concentration for adult bald eagles in the PRW watershed.

2. Study area

Bald eagle blood and feathers were sampled along approximately 81% of the coastline in Maine, U.S.A., from Casco Bay to Cutler (Fig. 1). We focused in particular on the Penobscot River watershed, which spans multiple habitats associated with the East Branch, West Branch and main branches of the Penobscot River and Penobscot Bay. The PRW was delineated using the HUC-6 hydrologic unit map boundary extracted from the USGS Watershed Boundary Dataset (W.B.D., 2016). The southern portion of the study area was delineated to include HUC-10 level subdrainages of Penobscot Bay, Belfast Bay, and the Bagaduce River. The PRW hosts just under one quarter of the bald eagle nesting territories statewide (based on a 2013 statewide survey, Maine Department of Inland Fisheries and Wildlife, unpubl. data). HoltraChem is located in Orrington in the lower Penobscot River, approximately 20 km downstream from the Veazie dam. The area just below the Veazie dam hosts the interface between freshwater inputs from above the dam and upstream tidal flows from Penobscot Bay. The river section between the Veazie dam and Penobscot Bay is generally referred to as the lower Penobscot River. Penobscot Bay contains extensive rocky forested shorelines and numerous coastal islands with varying degrees of development. Tidal waters from Penobscot Bay also flow into the Bagaduce River in northeastern Penobscot Bay. The West Branch of the Penobscot River originates in northwestern Maine, flowing through several large impounded lakes before joining the East Branch in Millinocket. Industrial paper mills once operated along the course of the river in towns of Millinocket, East Millinocket, Lincoln, Old Town, Brewer and Bucksport. Bald eagle nest sites sampled throughout inland Maine were generally located in forested lake, river, and island shorelines with varying degrees of human development. Reference nest sites sampled outside the PRW broadly spanned the Maine coastline and were generally located on coastlines and forested coastal islands in locations including but not limited to Casco Bay, tidal sections of the Sheepscot River, the Maine Coastal Island National Wildlife Refuge, and Acadia National Park.

3. Materials and methods

3.1. Assessing the influence of HoltraChem on Hg exposure in bald eagles

Habitat type is known to influence Hg exposure in Maine bald eagles (DeSorbo, 2007; Mierzykowski et al., 2013b; Welch, 1994). Since HoltraChem is located downstream from the interface zone between fresh and tidal waters, habitat type confounds upstream versus downstream approaches typically used in wildlife risk assessments (Jackson et al., 2011). For example, all but one nest site upstream from HoltraChem were associated with freshwater habitats, while all nests downstream from HoltraChem were situated in estuarine or marine habitats. Therefore, in order to assess whether discharges from HoltraChem may have increased Hg exposure in bald eagles in the lower Penobscot River and Penobscot Bay, Hg exposure in bald eagle nestlings raised in areas potentially exposed to Hg from HoltraChem (the potential Hg impact zone) was compared to that of nestlings sampled at reference sites outside the PRW along the Maine coastline (Fig. 1). The criteria for including bald eagle nest sites in the potential Hg impact zone were: (1) nest sites had to be located in tidal-influenced waters (estuarine or marine habitat types in our study; see

below), and (2) nest sites had to be located within the PRW boundary and therefore had potential for Hg exposure from HoltraChem. Sediment sampling confirmed that the I-395 bridge, roughly 6.5 km upstream from HoltraChem (Fig. 1), was the upstream limit of Hg contamination in the lower Penobscot River (Bodaly and Kopec, 2013; Yeager et al., 2018). The I-395 bridge was used as the northernmost boundary of potential Hg impact zone. Since Hg from HoltraChem could contaminate potential bald eagle prey in Penobscot Bay, the Penobscot watershed boundary was used to delineate the southern extent of the potential Hg impact zone. Given known tidal flow patterns in upper Penobscot Bay, it was also deemed possible that Hg from HoltraChem could enter the Bagaduce River Estuary; therefore, three nests in this area were also included in the potential Hg impact zone.

3.2. Habitat type assignments

To place Hg exposure in the lower Penobscot River into context within the entire watershed, nestling bald eagles throughout the PRW, including freshwater river and lake habitats not exposed to Hg from HoltraChem, were sampled. Bald eagle nesting territories in the PRW were categorized into four different habitat types: freshwater lakes (lakes hereafter), freshwater river, estuarine, and marine following previous studies in Maine (DeSorbo, 2007; Welch, 1994). Nest sites were assigned to habitat categories on the basis of a combination of the surrounding habitat associated with nest sites, geography, and tidal characteristics. Nesting territories were categorized as lakes if they were located on or immediately adjacent to a natural or impounded lake (Fig. 1). Nest sites associated with freshwater or run-of-river impoundments, and those along shorelines of the Penobscot River corridor (including the East and West Branch sections) north of Bangor/Brewer were categorized as freshwater river sites. The nest site immediately below the Veazie Dam (sampled in 2006 and 2007) marks the southernmost periphery of the river reach categorized as freshwater river. River-based nest sites between the I-395 bridge and the southern end of Verona Island, and also three nests in the Bagaduce River were categorized as estuarine on the basis that they receive variable amounts of both marine and freshwater inputs. Nest sites in Penobscot Bay within the PRW boundary were categorized as marine sites. These habitat delineations are comparable to those used in related studies within the Penobscot River ecosystem (Bodaly et al., 2018, 2009b, 2008; Call, 2015).

Breaches or removals of dams along the Penobscot River (e.g., Great Works in 2012, Veazie Dam in July–November 2013), and fish lift installations (e.g., Milford in 2015, Howland Bypass in 2016) related to ongoing river restoration efforts in the Penobscot River (Call, 2015) did not complicate habitat assignments or data interpretations in this study since all sampling along the corridor and the majority of sampling overall, occurred prior to dam removals or breaches. For example, 97.5% (119 of 122) of annual visits to sample nestlings in the PRW occurred during 2003–2013, prior to the removal of the Veazie Dam that initiated the most notable ecological changes along the corridor by enabling passage of diadromous fish. No nestlings were sampled in the PRW in 2014, and only three lake nests (13–18 km from the Penobscot River Corridor) were visited for nestling sampling in 2015.

3.3. Nestling bald eagle handling and tissue sampling

Eighty-eight bald eagle nesting territories throughout the PRW and 43 nesting territories along the coast outside of the PRW were visited to collect nestling tissue samples (Fig. 1). Sampling in the PRW occurred between 2003 and 2015 as part of ongoing efforts to evaluate Hg exposure in freshwater-nesting bald eagles statewide (DeSorbo and Evers, 2007; DeSorbo, 2007; DeSorbo et al., 2009), and sampling efforts toward the PRMS that primarily occurred in 2007 (Bodaly et al., 2018). Reference sites outside the PRW along the Maine coast were sampled in 2007–2013 as reference sites for the PRMS or as a part of a coastal-focused bald eagle contaminants study (Mierzykowski et al., 2013b).

Prior to field visits, nests with young eaglets >4 weeks of age were identified by aerial reconnaissance using a fixed-wing aircraft. Sampling visits generally occurred when nestlings were approximately 6 weeks of age. Nestlings were banded with U.S. Geological Survey (USGS) and colored leg bands, and sampled on the ground before being returned to the nest. Blood was sampled from the cutaneous ulnar vein in the wing of nestlings using 21–25 gauge butterfly™ needles attached to heparinized evacuated test tubes. Blood was collected in heparinized capillary tubes (ca. 75 μL each) and 5–7 mL heparinized test tubes. Samples were placed into protective cases in a cooler filled with ice packs and were frozen to <30 °F, typically within 10 h of collection. Approximately 4–6 breast feathers were collected from of each nestling using wire cutters or stainless steel scissors, placed into envelopes, and stored at room temperature. Morphometrics (e.g., length of bill, culmen, footpad, eighth primary, tarsus width) were recorded during processing and nestlings were aged using the eighth primary following Bortolotti (1984a). Bald eagle banding and sampling were conducted under authorization of permits issued by Maine Department of Inland Fisheries and Wildlife (MDIFW) and the USGS Bird Banding Lab.

3.4. Adult bald eagle sampling

To provide perspectives on Hg exposure and toxicological risk in adult bald eagles in the PRW, blood samples were also collected from adult bald eagles captured in their nesting territories. All adult eagles were captured on lakes between May and July 2015 except for one adult that was captured in September (and averaged with another adult captured at the same nesting territory in July). Sampling locations were selected as part of an unrelated bald eagle tracking study in which adults associated with confirmed active nests were targeted for capture. Adult bald eagles were captured using floating fish snares as described by Jackman et al. (1993). After capture, adults were hooded and sampled for blood as described above for nestlings.

3.5. Statistical analyses

The Shapiro-Wilk test and visual data inspection were applied to assess normality of datasets. Both blood Hg and feather Hg data displayed log-normal distributions, thus $\log_{10}$(blood Hg) and $\log_{10}$(feather Hg) transformations were used in all statistical analyses. To evaluate whether bald eagle tissue Hg concentrations were elevated due to the potential influence of Hg discharges by HoltraChem, a *t*-test was applied to compare tissue Hg concentrations between nestlings sampled in the potential Hg impact zone to those sampled at reference sites. To evaluate the influence of habitat type (marine, estuarine, freshwater river, lake) on bald eagle tissue Hg concentrations within the PRW, nestling blood and feather Hg concentrations were averaged for same-year siblings and for nestlings sampled in multiple years within nesting territories. A Bartlett's test was applied to ensure variances were equal among groups and then a one-way ANOVA was used to test for differences in nestling blood and feather Hg concentrations among habitat groups. The Tukey's HSD test was used to make pairwise comparisons between habitat groups. A Model II regression was applied to evaluate the relationship between bald eagle nestling blood and feather Hg concentrations, and to model feather Hg concentrations from blood Hg values. Model II regression is preferable to Model I regression when both variables are measured with error (Sokal and Rohlf, 2003). To assess whether nestling blood or feathers provided better data for detecting differences in bald eagle Hg exposure among habitat types, a power analysis was conducted for a one-way ANOVA using observed means and standard deviations for $\log_{10}$(blood Hg) and $\log_{10}$(feather Hg). The power analysis assumed balanced sampling from the four habitat types and $\alpha = 0.05$. All statistical tests were performed using JMP 13.1.0 (SAS, 2016), with the exception of the Model II regression, which was performed in R, version 3.3.2 (R Core Team, 2016) using the 'lmodel2' package. Geometric means and asymmetric standard

deviations are presented in text and tables, back-transformed from analyses of log10-transformed Hg data. Results of statistical tests were considered significant at $\alpha = 0.05$.

3.6. Laboratory analysis

Data used for this study were generated at several laboratories. Although there were minor variations in sample preparation and analytical methods, all analyses included quality control samples to allow evaluation of accuracy and precision. Blood samples were analyzed at the BRI Toxicology Laboratory at Biodiversity Research Institute (Portland, Maine) and the Trace Element Research Laboratory (TERL), Texas A & M University (College Station, TX) with a Milestone DMA 80 Direct Mercury Analyzer using thermal decomposition and atomic absorption spectrometry (EPA method 7473); at Laboratory and Environmental Testing (LET, Columbia, MO) with a Perkin Elmer cold vapor atomic absorption spectrometer (EPA method 245.6), and at Battelle Marine Sciences Laboratory (Battelle; Sequim, Washington) with a Tekran Model 2500 cold vapor atomic fluorescence spectrometer (EPA method 1631e). One whole breast feather sample per bird was analyzed at the BRI Toxicology Laboratory and at the Savannah River Ecology Lab (SREL) at the University of Georgia (Aiken, SC) using the same methods as described above for blood. All blood samples were analyzed as whole blood on a wet weight (ww) basis and are reported in parts per million (μg/g). At Battelle, feathers were washed with dilute baby shampoo (shown to contain no mercury), rinsed with deionized water, and allowed to dry prior to analysis. Breast feathers were rinsed with 1% Alconox Liquinox® critical cleaning liquid detergent dissolved in 18.2 MΩ deionized water and rinsed with 18.2 MΩ deionized water at SREL prior to analysis. Breast feathers were inspected for surface debris before being analyzed unwashed and whole at BRI. Feather mercury results were reported in parts per million (μg/g) on a fresh weight (fw) basis. Total Hg was measured as a proxy for MeHg, since total Hg has been consistently found to be >95% MeHg in bird blood and feathers (Evers et al., 2005; Thompson et al., 1990).

For all samples analyzed, each laboratory generally included 2 analytical blanks, ≥1 sample replicate, ≥1 spiked sample and certified reference materials (CRMs; National Research Council, Canada and Joint Research Centre, European Union) for every 20–30 samples. While there is no CRM specific to feather, the CRMs used for blood and feather are commonly used in a wide variety of published toxicological studies. All labs reported the relative percent difference for duplicates to be <10%. All samples were above method detection limits for both blood (BRI Toxicology Laboratory = 0.001 μg/g; LET = 0.0210 μg/g; TERL = 0.0014–0.0025 μg/g; Battelle = 0.000495 μg/g) and feathers (BRI Toxicology Laboratory = 0.001 μg/g; SREL = 0.28 μg/g; Battelle = 0.00053 μg/g). Recoveries of CRMs for blood analyses were as follows: BRI Toxicology Laboratory (DOLT-4 = 102%, and DORM-3 = 100%), LET (DOLT-3 = 113% and TORT-2 = 111%), TERL (DORM-2 = 94–100%; DOLT-3 = 99%–102%; DOLT-2 = 100–102%). Recoveries of CRMs for feather analyses were as follows: BRI Toxicology Laboratory (DOLT-4 = 101%, DOLT-5 = 104%, DORM-3 = 99%, and BCR-463 = 99%), SREL (TORT-2 = 105%) and Battelle (DOLT-2 = 103%). Multiple laboratories used in this study have provided highly comparable results in other studies when measuring total Hg in avian tissues (Cristol et al., 2012; Meattey et al., 2014; Savoy et al., 2017). Given all samples analyzed in this study were above method detection levels and all laboratories met USEPA quality assurance standards (USEPA, 2007), Hg results are considered comparable across laboratories.

4. Results

A total of 221 bald eagle nestlings were sampled from 131 nest sites (88 in the PRW, and 43 reference nest sites) in this study. Nine adult bald eagles from eight nesting territories were additionally sampled on lakes in the PRW.

4.1. Hg exposure in bald eagle nestlings in the potential Hg impact zone

Bald eagle nestlings in the potential Hg impact zone had higher tissue Hg concentrations than those from reference sites. The geometric mean blood Hg concentration for bald eagle nestlings sampled in marine and estuarine habitats in the potential Hg impact zone ($\bar{x}$: 0.21 μg/g; lower, upper SDs: 0.12, 0.38 μg/g; range: 0.073–0.60 μg/g; Fig. 2) was significantly higher than the mean from reference sites ($\bar{x}$: 0.13 μg/g, lower, upper SDs: 0.069, 0.23 μg/g; range: 0.031–0.42 μg/g) ($p = 0.0054$, $t_{57} = -2.89$, $n = 59$). Similarly, geometric mean breast feather Hg concentrations were significantly higher in pooled marine and estuarine habitats in the potential Hg impact zone ($\bar{x}$: 6.4 μg/g; lower, upper SDs: 4.3, 9.5 μg/g; range: 3.0–16.1 μg/g) compared to reference sites ($\bar{x}$: 4.0 μg/g, lower, upper SDs: 2.2, 7.2 μg/g; range: 1.2–19.3 μg/g) ($p = 0.0057$, $t_{53} = -0.29$, $n = 55$).

4.2. Habitat-based differences in bald eagle Hg exposure within the PRW

Geometric mean Hg concentrations in nestling blood and feathers differed significantly among lake, freshwater river, estuarine and marine habitat types within the PRW ($\log_{10}$[blood Hg]: $p < 0.0001$, $F_{3, 84} = 20.2$; $\log_{10}$[feather Hg]: $p < 0.001$, $F_{3, 79} = 39.5$). Mean Hg concentrations in both tissue types increased among habitat types when moving from marine habitats inland as follows: marine = estuarine < freshwater river < lake; however, not all mean pairs differed statistically (Fig. 3, Table 1). Significant differences in mean Hg concentrations among habitat types were more common in breast feathers than blood in pairwise comparisons. In breast feathers, only mean Hg concentrations in marine and estuarine habitat types did not differ ($p > 0.05$). Mean Hg concentrations of feathers sampled at marine and estuarine sites were both significantly lower than means for freshwater rivers and lakes, which differed from each other ($p < 0.05$). Mean Hg concentrations in nestling blood did not differ between marine and estuarine habitats, and were also similar between lakes and rivers ($p > 0.05$). Nestlings from the freshwater habitats (lake and river), however, did have higher mean blood Hg concentrations than those in marine and estuarine habitats ($p < 0.0001$). The highest Hg concentrations in individual bald eagle nestling tissues were observed at nest sites associated with freshwater-based habitats throughout the PRW, rather than in the potential Hg impact zone.

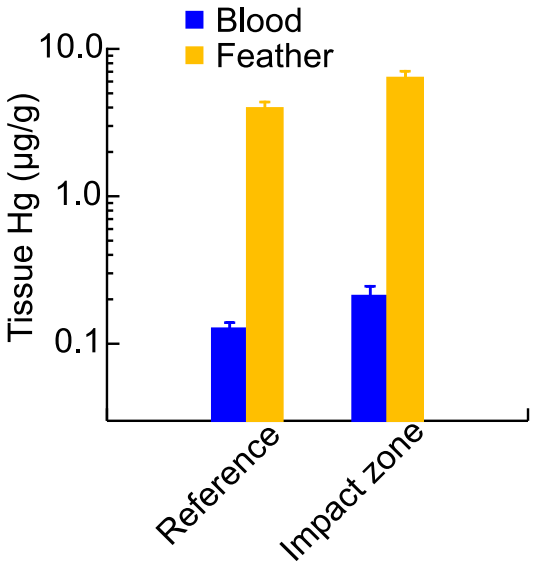

Fig. 2. Geometric mean blood (μg/g, ww) and feather (μg/g, fw) Hg concentrations in nestling bald eagles sampled within the potential Hg impact zone (n = 16) and at reference sites along the Maine coast (n = 43). Whiskers on bars indicate standard error.

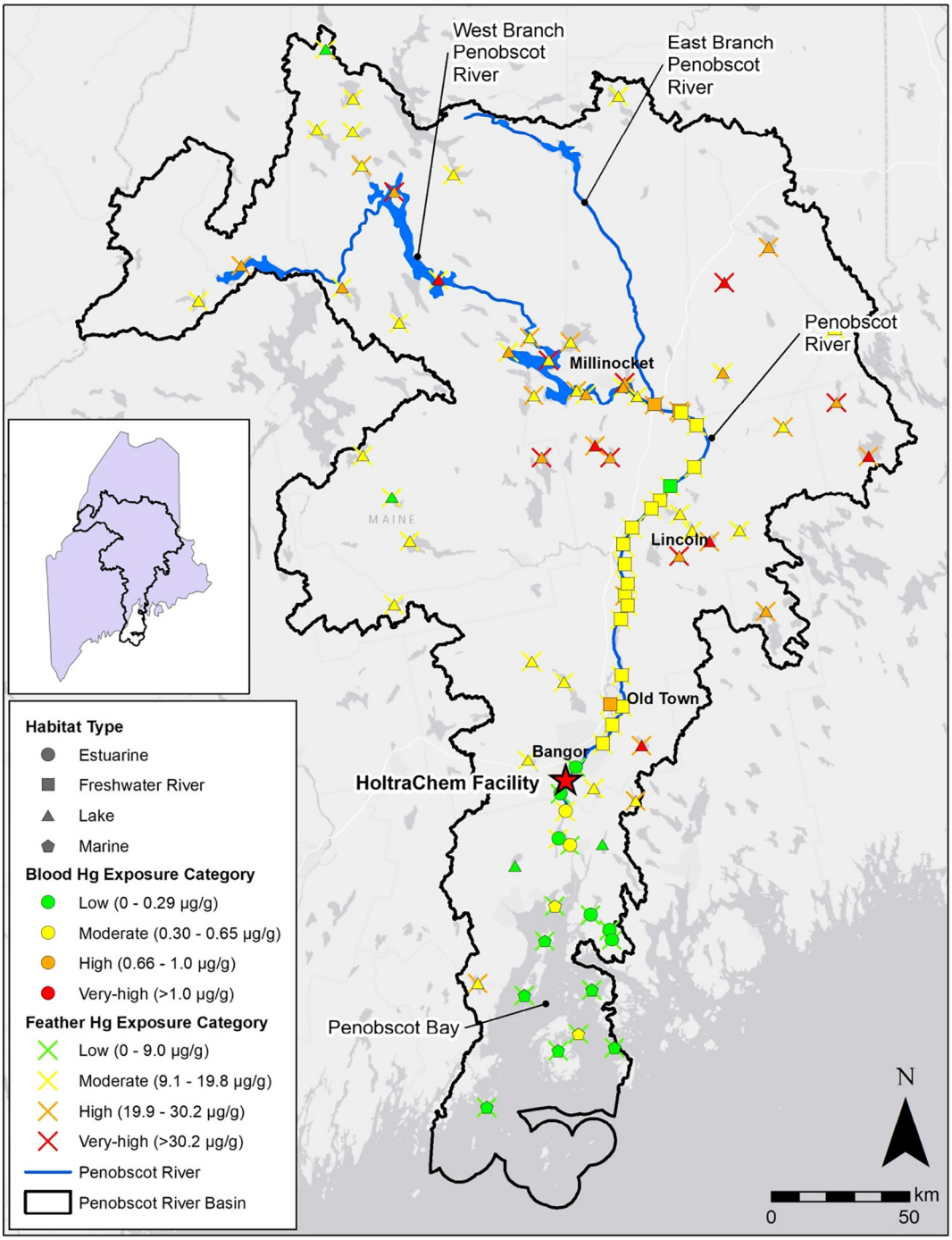

Fig. 3. Mercury concentrations in blood and breast feathers of bald eagle nestlings sampled in four different habitat types within the Penobscot River Watershed, Maine. Estuarine and marine nests comprise sample sites in the potential Hg impact zone (see Fig. 1). Blood Hg categories delineated based upon Kenow et al. (2007) and DeSorbo et al. (2009); feather Hg risk categories calculated from blood Hg risk categories using equation described in Section 4.3. (online version in color).

Table 1
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 four habitat types in the Penobscot River Watershed.[a]

Habitat type	Blood µg/g, ww				Breast feather µg/g, fw			
	n	Mean	SD	Range	n	Mean	SD	Range
Marine	8	0.18 A	0.09, 0.37	0.07–0.60	8	6.3 A	5.0, 8.0	4.18–8.43
Estuarine	8	0.25 A	0.17, 0.37	0.12–0.45	8	6.4 A	3.7, 11.0	2.95–16.1
Freshwater river	21	0.45 B	0.33, 0.62	0.27–0.79	21	15.2 B	11.8, 19.5	9.35–23.6
Lake	51	0.57 B	0.36, 0.90	0.13–1.51	46	19.6 C	13.4, 28.6	8.75–46.9
Overall	88	0.45	0.25, 0.80	0.07–1.51	83	14.7	8.5, 25.5	2.95–46.9

[a] Blood and feather Hg concentrations were averaged for siblings and at nests sampled in >1 yr. Means within columns sharing the same letter were not significantly different (Tukey's multiple comparison, $p < 0.05$; *t*-test ($p < 0.05$)). Geometric means and asymmetric standard deviations back-calculated from Log_{10}(Blood Hg) and Log_{10}(Feather Hg) data.

4.3. Comparing the biomonitoring value of nestling bald eagle blood vs. feathers

Log_{10}-transformed Hg concentrations in breast feathers were significantly related to log_{10}-transformed Hg concentrations in blood of bald eagle nestlings sampled in the PRW and at reference sites ($p < 0.01$, $R^2 = 0.72$; $n = 176$; $log_{10}[\text{feather Hg}] = 1.48 + 0.98 * log_{10}[\text{blood Hg}]$; Fig. 4). Power analysis of the log_{10}(blood Hg) and log_{10}(feather Hg) data for eagle nestlings from the four habitat types in the PRW revealed that feather Hg data had 31% greater statistical power to detect differences among the four habitats than blood Hg data ($\beta_{feather} = 0.915$, $\beta_{blood} = 0.602$, $n = 12$).

4.4. Hg exposure in adult bald eagles in the PRW

Concentrations of Hg in blood of the nine adult bald eagles sampled at lakes in the PRW ranged from 1.86–6.73 μg/g. The geometric mean blood Hg concentration in adults was 3.14 μg/g (upper and lower asymmetric SDs: 2.07, 4.79 μg/g; $n = 8$).

5. Discussion

Mercury concentrations in bald eagle nestlings sampled in the PRW demonstrate the dominant role that site- and habitat-specific factors play in influencing Hg bioavailability to biota, including wide-ranging species like bald eagles. While our findings demonstrate that bald eagle nestlings in the potential Hg impact zone had higher tissue Hg concentrations compared to those in reference areas, these levels were still notably lower than those found in nestlings associated with freshwater river and lake habitats unaffected by Hg from HoltraChem.

5.1. Influence of HoltraChem on bald eagle Hg exposure in the potential Hg impact zone

Bald eagle nestlings raised in both marine and estuarine habitats in the lower Penobscot River exhibited significantly higher blood and feather Hg concentrations than those sampled at reference sites along the Maine coast. Evidence indicating that Hg concentrations are elevated in bald eagles in the potential Hg impact zone is consistent with findings from sediment, wetlands, shellfish, fish, and several bird species in the lower Penobscot River, estuary, and Penobscot Bay sampled during the PRMS (Bodaly et al., 2018, 2008; Rudd et al., 2018; Sullivan and Kopec, 2018; Turner et al., 2018; Yeager et al., 2018). The spatial patterns of Hg contamination in several species of fish and birds, and particularly those found in sediment, are consistent with a large source of Hg from a location below the Veazie Dam (Bodaly et al., 2018; Yeager et al., 2018). While sediment sampling revealed evidence of historic anthropogenic Hg inputs into river reaches upstream from the Veazie dam, Hg concentrations in upstream sediments were dramatically lower than those in the potential Hg impact zone (Bodaly et al., 2018; Yeager et al., 2018).

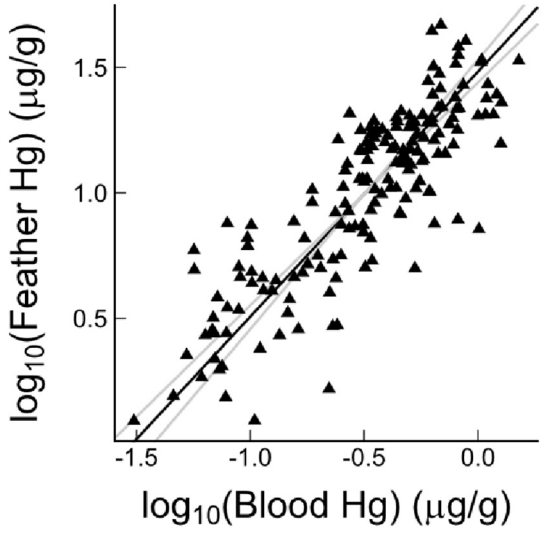

Fig. 4. Model II regression of log_{10}-transformed blood and log_{10}-transformed breast feather Hg concentrations of bald eagle nestlings from the Penobscot River watershed and the Maine coast. Gray lines indicate 95% confidence limits.

Evidence that Hg concentrations are elevated in bald eagle nestlings in the potential Hg impact zone compared to reference sites suggests that historic Hg inputs from HoltraChem continue to contaminate the food web upon which bald eagles and other piscivores depend in the Penobscot River ecosystem. Sediment sampling confirmed that MeHg, confirmed to be high in surface sediments, is positively related to concentrations of inorganic (industrial) Hg, revealing the high likelihood that areas contaminated with Hg are contributing MeHg to the foodweb (Rudd et al., 2018). Although current levels of Hg loading into the Penobscot River are relatively low upstream and downstream from HoltraChem (Turner et al., 2018), the twice daily mixing of tidal and fresh waters in the river are considered important in the release and methylation of Hg in surface sediments and redistribution to biota in the lower Penobscot River and Penobscot Bay (Rudd et al., 2018).

5.2. Factors influencing Hg bioavailability to bald eagles in the PRW

Notable geographic and habitat-based differences in bald eagle Hg exposure patterns were found throughout the PRW (Fig. 3). The overall pattern of increasing Hg concentrations in tissues sampled from marine to freshwater settings (marine = estuarine < freshwater river < lakes) has been previously reported in Maine bald eagles (Welch, 1994) and other piscivores such as belted kingfishers *Megaceryle alcyon* (Evers et al., 2005). Evidence supporting lower Hg exposure in marine versus freshwater settings has been found in other piscivores, including wood storks (*Mycteria americana*; Gariboldi et al., 2001), wading birds (Frederick et al., 2002) and river otter (*Lontra canadensis*; Yates et al., 2005). Differences in bald eagle Hg exposure have also been observed between freshwater habitats in the Great Lakes (Best et al., 1994; Bowerman et al., 1995; Colborn, 1991) and in Maine (DeSorbo, 2007; DeSorbo et al., 2009). In our study, Hg concentrations in the blood and feathers of bald eagle nestlings at lakes, many of which are remote in the PRW, were roughly three times higher than the mean concentrations for nestlings sampled in the potential Hg impact zone (Table 1). Bald eagle sampling elsewhere in Maine indicates that Hg contamination in freshwater habitats is not unique to the PRW, but evident in other watersheds including the Androscoggin, Saint Croix, and Saint John River Basins (DeSorbo, 2007). This finding emphasizes how factors related to location and habitat type can potentially have a stronger influence on Hg exposure in bald eagles than the presence of a sizeable point source.

5.2.1. Atmospheric deposition and Hg methylation

Atmospheric inputs are considered the primary source of Hg to fish and wildlife in numerous remote areas throughout northeastern North America (Burgess and Hobson, 2006; Evers et al., 2007; Evers and Clair, 2005; Kamman et al., 2005; Rimmer et al., 2005). West to east directional wind patterns facilitate the transport of atmospheric Hg pollution generated in New England, the Midwestern U.S. and from global sources to northeastern North America (Driscoll et al., 2013; Mason et al., 2005; Vanarsdale et al., 2005). Once deposited on the landscape, a wide variety of site- and habitat-specific factors influence MeHg production and bioavailability to biota (Driscoll et al., 2013, 2007; Eagles-Smith et al., 2016). In a recent synthesis, differences in the distribution of organic and inorganic Hg sources, climate, differential rates of MeHg production and bioaccumulation, land cover factors, and water level management were important factors heterogeneously influencing

ecological Hg risk across the landscape in the Western U.S. (Eagles-Smith et al., 2016). Complex and lengthy food webs also promote the biomagnification of Hg (Atwell et al., 1998; Kidd et al., 1995) that is especially relevant to apex predators such as bald eagles. Regular wetting and drying cycles occur at natural lakes, impoundments, and both freshwater and tidally influenced river habitats in the PRW (Turner et al., 2018). Findings in this study demonstrate that the influence of a large Hg discharge into the lower Penobscot River was of lower importance to Hg exposure in bald eagle nestlings compared to the combined influence of atmospheric Hg inputs and site-specific factors influencing MeHg bioavalability in freshwater ecosystems.

5.2.2. Dietary influences on Hg exposure

Bald eagles exploit a diverse range of food resources, commonly shifting dietary habits by season and region (Buehler, 2000; Stalmaster, 1987). Differences in prey trophic level and overall dietary composition likely drive the Hg exposure patterns observed among habitats in this study. Bald eagles along the coast of Maine, particularly at offshore islands, feed heavily on birds, while fish are foremost in diets at inland freshwater lakes and rivers (Todd et al., 1982, CD, personal observation). Mercury exposure varies widely among different fish and bird species according to trophic level, habitat type, waterbody, waterbody type, season and numerous other factors (Evers et al., 2005; Kamman et al., 2005; Kidd et al., 1995; Munthe et al., 2007; Sullivan and Kopec, 2018), confounding comparisons of bald eagle Hg exposure by any single factor. For example, three freshwater fish species that comprised 84% of the diet of inland bald eagles in Maine, chain pickerel (*Esox niger*), brown bullhead (*Ictalurus nebulosus*), and white sucker (*Catostomus commersoni*) (Todd et al., 1982), span the Hg exposure range of freshwater fish in the Northeastern U.S., with white sucker among the lowest (mean fillet Hg: 0.19 μg/g), and chain pickerel among the highest (mean fillet Hg: 0.55 μg/g) (Kamman et al., 2005). Mercury concentrations in freshwater fish in Maine commonly exceed human health fish consumption benchmarks established by the Great Lakes Fish Advisory Workgroup (>0.05 to 0.11 μg/g, two fish meals per week; >0.11 to 0.22 μg/g, one fish meal per week; 0.22 to 0.95 μg/g, one fish meal per month; and no fish consumption >0.95 μg/g; Lepak et al., 2016). Mean fillet Hg concentrations in numerous freshwater fish species sampled in northeastern North America (yellow perch *Perva falvescens*, 0.44 μg/g; largemouth bass *Micropterus salmoides*, 0.54 μg/g; smallmouth bass *microperus dolomieui*, 0.59 μg/g; white perch *Morone americana*, 0.72 μg/g; Kamman et al., 2005) commonly exceed dietary prey screening benchmarks for piscivorous birds (0.1–0.4 μg/g; Depew et al., 2012).

Sampling of birds in the PRMS, some of which are potential prey for bald eagles, demonstrated that Hg concentrations are elevated in some species across multiple foraging guilds in the PRW (Bodaly et al., 2009a; Kopec et al., 2018b). Mercury concentrations in eggs of piscivores, such as double-crested cormorants and black guillemots, exceeded levels associated with 20% reduced reproductive success (i.e., 0.65 μg/g ww; Evers, 2018), while Hg concentrations in blood or egg samples collected from invertivores such as shorebirds, Virginia rails (*Rallus limicola*) and several passerines regularly exceeded levels associated with 20% reduced reproductive success in invertivores (i.e., 1.2 μg/g, ww in adult bird blood and 0.2 μg/g, ww in eggs; Evers, 2018). Mean Hg concentrations in muscle of black ducks, an invertivore hunted in the lower Penobscot River, reached 0.82 μg/g ww by the late fall, prompting regulatory agencies to issue health advisories limiting human consumption of duck muscle in the lower Penobscot River (Sullivan and Kopec, 2018), while lower trophic level invertivores such as common eiders (*Somateria mollissima*), exhibit relatively low Hg exposure in Penobscot Bay (Goodale et al., 2008; Meattey et al., 2014).

Stable isotope analyses of bald eagle feathers sampled in the Penobscot River and Penobscot Bay indicated that bald eagle nestlings generally consumed freshwater and marine-based protein sources consistent with the habitat type in which they were raised (Call, 2015). Inshore bald eagle pairs along coastal Maine have ready access to "river herring" (alewives *Alosa pseudoharengus* and blueback herring *Alosa aestivalis*) (Todd et al., 1982), diadromous fish with relatively low Hg concentrations (B. Mower, Maine Department of Environmental Protection, pers. com.) as compared to many bald eagle prey alternatives. Bald eagle diets in the PRW dominated by freshwater fish likely expose individuals to higher Hg concentrations than pairs in the lower river and bay impacted by the chlor-alkali plant, which may moderate their exposure to Hg by consuming low Hg prey such as river herring (Call, 2015; Todd et al., 1982). Continuing increases in river herring populations in response to historic restoration efforts in the Penobscot River should increase the availability of low Hg prey to bald eagles throughout much of the PRW in the future (Call, 2015; MDMR, 2017).

5.3. Population comparisons of Hg in nestling bald eagles

Within habitats, bald eagles in the PRW generally exhibit higher Hg concentrations than most breeding populations elsewhere (see references in Table 2, Table 3). Mercury concentrations in bald eagle tissues sampled at reference sites in this study were similar to those sampled in Newfoundland, Canada (Dominguez et al., 2003) and in coastal/inland South Carolina (Jagoe et al., 2002). Mercury concentrations in nestling tissues sampled in the potential Hg impact zone were more than two times higher than nestlings sampled in these regions. In our literature search, only nestlings in the highly contaminated Columbia River Estuary in 1980–1987 had higher blood Hg concentrations than nestlings sampled in estuarine habitats in the PRW (Anthony et al., 1993; Table 2).

In freshwater river habitats, the mean breast feather Hg concentration in PRW nestlings was roughly three and five times higher than that of nestlings sampled in the Mississippi River and the Saint Croix National Scenic Riverway, respectively (Dykstra et al., 2010). The mean blood Hg concentration of nestlings sampled in freshwater river habitat was 27% higher in the PRW than nestlings sampled in northwestern Wyoming (lakes and rivers combined) and 2–4.5 times higher than the mean for nestlings sampled in southeastern and southwestern Montana (Carlson et al., 2012; Harmata, 2011) (Table 2, Table 3). Nestlings raised on lakes in the PRW generally exhibited higher tissue Hg concentrations than bald eagle populations in Florida, South Carolina, Washington State (Klamath basin and the Cascade Lakes), New York State (excluding the Catskill Park), the Great Lakes region, British Columbia (reference lakes in the Hg-rich Pinchi Lake region) and Wyoming (Bowerman et al., 1994; Carlson et al., 2012; DeSorbo et al., 2008; Jagoe et al., 2002; Pittman et al., 2011; Weech et al., 2006; Wiemeyer et al., 1989; Wood et al., 1996) (Table 2, Table 3). Mercury exposure in nestlings from lakes in the PRW were most comparable to three populations with significant and well-known Hg pollution issues: (1) Voyageurs National Park in 1985–1989, prior to water level stabilization (Bowerman et al., 1994; Pittman et al., 2011), (2) Pinchi Lake, a large lake in British Columbia associated with a Hg mine (Weech et al., 2006, 2004), and (3) the Catskill Park region in southeastern New York State, a well-established biological Hg hotspot (DeSorbo et al., 2008; Evers et al., 2007; Townsend et al., 2014).

5.4. Population comparisons of Hg in adult bald eagles

While Hg exposure in bald eagle nestlings represents an index of adult Hg exposure (Weech et al., 2006), Hg concentrations are infrequently reported in territorial adults. Direct data on Hg exposure in adults is important because adverse effect thresholds for Hg are predominantly established in the adult age class due to the confounding influence of body and feather growth on circulating Hg levels in nestlings (Ackerman et al., 2016; Evers et al., 2008, 2005). The geometric mean adult blood Hg concentration for lake-nesting bald eagles in the PRW in our study (3.16 μg/g) is similar to the mean concentration found in seven adult eagles sampled in the Columbia River Estuary ($\bar{x}$: 3.07 μg/

Table 2
Arithmetic mean, standard deviation, geometric mean, and range of total Hg concentrations in blood of bald eagle nestlings sampled throughout North America.

Year	Region	Habitat type	Blood Hg (μg/g ww)[a]					Study
			n[b]	Mean	SD	GeoMean	Range	
Marine-influenced (estuarine and marine)								
1980–87	Columbia River Estuary, Oregon/Washington	Estuarine	15 [c]	0.47			0.19–1.4	Anthony et al., 1993
2003–15	*Maine, Penobscot River Watershed*	*Estuarine*	*8*	*0.27*	*0.10*	*0.25*	*0.12–0.45*	*This study*
2003–15	*Maine, Penobscot River Watershed*	*Marine*	*8*	*0.23*	*0.18*	*0.18*	*0.07–0.60*	*This study*
2007–13	*Maine (reference for PRW)*	*Marine/Estuarine*	*43*	*0.15*	*0.089*	*0.13*	*0.031–0.42*	*This study*
1991–92	Maine, statewide	Estuarine	9–10	0.15	0.061	0.12	0.014–0.34	Welch, 1994[d]
1998–99	South Carolina	Marine/Inland	8–10	0.10	0.073		0.02–0.25	Jagoe et al., 2002[d]
1996–97	Newfoundland	Marine	23			0.087	0.05–0.25	Dominguez et al., 2003
1991–92	Maine, statewide	Marine	22–25	0.096	0.073	0.079	0.016–0.46	Welch, 1994[d]
Freshwater river								
2003–15	*Maine, Penobscot River Watershed*	*River*	*21*	*0.48*	*0.16*	*0.45*	*0.27–0.79*	*This study*
2004–06	Maine, statewide	River	36			0.39	0.11–0.98	DeSorbo, 2007
2007–08	Wyoming (northwestern)	River/Lake	18	0.37	0.22		0.11–0.80	Carlson et al., 2012
1991–92	Maine, statewide	River	5–6	0.28	0.12	0.26	0.15–0.58	Welch, 1994[d]
2007–08	Montana (southeastern)	River	15	0.22	0.17		0.10–0.85	Carlson et al., 2012
2006–08	Montana (southwestern)	River	17[c]			0.10		Harmata, 2011
Lake								
1979–81	Oregon, (western)	Lake	82[c]			1.2	nd–4.2	Wiemeyer et al., 1989
2003–15	*Maine, Penobscot River Watershed*	*Lake*	*51*	*0.62*	*0.27*	*0.57*	*0.13–1.51*	*This study*
2000–02	British Columbia, Pinchi Lake (Hg mine)	Lake	12	0.57	0.16		0.37–0.79	Weech et al., 2006
1998–06	New York, (southeastern), Catskill Park	Lake	12	0.52	0.25			DeSorbo et al., 2008
2004–06	Maine, statewide	Lake	112			0.52	0.084–1.51	DeSorbo, 2007
1991–92	Maine, statewide	Lake	14–16	0.58	0.30	0.48	0.070–1.46	Welch, 1994[d]
2000–02	British Columbia, (central), multiple sites	Lake	31	0.27	0.083		0.12–0.60	Weech et al., 2006[e]
1998–06	New York, statewide	Lake	19	0.26	0.16			DeSorbo et al., 2008
1979–81	Washington (western)	Lake	9[c]			0.23	0.075–0.65	Wiemeyer et al., 1989
1991–93	Florida (central)	Lake (mesotrophic)	21[c]	0.20		0.17	0.02–0.61	Wood et al., 1996
1991–93	Florida (central)	Lake (eutrophic)	26[c]	0.13		0.11	0.04–0.48	Wood et al., 1996

[a] Table entries sorted from largest to smallest mean value within habitat groupings.
[b] Sample size represents number of nesting territories unless noted otherwise.
[c] Sample size equals number of individual nestlings sampled.
[d] Annual means and standard deviations averaged. Sample size shows range of annual sample sizes.
[e] Mean represents the average Hg concentration of four multi-territory lakes used as references to Pinchi Lake. Standard deviations averaged.

g; range: 1.3–4.10 μg/g; Anthony et al., 1993), higher than that reported for seven adult bald eagles sampled in the Klamath Basin and Cascade Lakes region of Oregon ($\bar{x}$: 2.3 μg/g; range: 1.1–4.8 μg/g; Wiemeyer et al., 1989), within the range of means for reference lakes in the Hg-rich Pinchi Lake region, British Columbia with varying degrees of natural and anthropogenic Hg inputs (2.0 μg/g–4.1 μg/g; Weech et al., 2006), but was roughly half that of the mean for three adult bald eagles sampled on Pinchi Lake ($\bar{x}$: 6.7 μg/g; range: 4.7–9.4 μg/g; Weech et al., 2006).

5.5. Interpreting Hg exposure risk

Mercury exposure at concentrations commonly found in bald eagle prey has been linked to a wide variety of subclinical and acute negative impacts on birds including those related to health, physiology, neurological function, immune function, behavior and reproduction (Ackerman et al., 2016; Chan et al., 2003; Evers, 2018; Fuchsman et al., 2016; Scheuhammer et al., 2007; Wiener et al., 2003). While elevated dietary exposure to Hg has been linked to reduced productivity in common loon populations in New York, New England, Wisconsin, Nova Scotia and Ontario (Barr, 1986; Burgess and Meyer, 2008; Evers et al., 2008; Schoch et al., 2014), only a limited number of studies have detected evidence indicating that Hg adversely affects bald eagles. Most previous assessments of Hg effects on bald eagles are complicated however, by the fact that other "legacy" contaminants such as DDE and PCBs confounded evaluations of the effects of Hg (Bowerman et al., 1994; Wiemeyer et al., 1993, 1984). Rutkiewicz et al. (2011) reported 14–27% of eagles sampled in the Great Lakes were exposed to Hg at concentrations associated with subclinical neurological damage. This finding has implications for Maine's bald eagle population since bald eagles in freshwater river and lake habitats in Maine are exposed to similar or higher concentrations of Hg to those in the Great Lakes (Table 2, Table 3). Unpublished studies in Maine detected negative correlations between productivity and Hg exposure in lake-nesting bald eagles in Maine (DeSorbo and Evers, 2007; DeSorbo et al., 2009) during more recent periods, when legacy contaminants had generally declined to levels below impact thresholds (Mierzykowski, 2010, 2006; Mierzykowski et al., 2013a, 2013b, 2011; Mierzykowski and Carr, 2002).

This study provides the first measure of Hg in adult bald eagle blood in Maine. While adverse effects from Hg have been documented as low as 0.2 μg/g in adult bird blood, 3.0 μg/g has been associated with severe physiological impairment on health and reproduction in adult birds, while 4.0 μg/g is linked to with severe physiological impairments and complete reproductive failure (Ackerman et al., 2016). Evers et al. (2008) found adult common loons with blood Hg concentrations ≥3.0 μg/g produced 41% fewer fledged young than breeding loons with blood Hg concentrations ≤1.0 μg/g. Burgess and Meyer (2008) found Hg imposed an upper limit on loon productivity, with maximum productivity dropping by 50% when adult loon blood contained 4.3 μg/g Hg, and reproductive failure occurred when adult loon blood contained 8.6 μg/g Hg. Of the adult bald eagles sampled in the PRW in our study, 55% and 33% had blood Hg concentrations >3.0 μg/g and >4.0 μg/g, respectively. Continued feather growth in adult eagles during the breeding season and an enhanced capacity of bald eagles to demethylate MeHg may offer protective mechanisms against the toxicological effects of Hg not afforded to some species such as common loons (Scheuhammer et al., 2008).

Several dosing studies provide perspectives on adverse impacts associated with elevated blood Hg concentrations in developing nestling birds. Concentrations of 0.66 μg/g Hg in blood have been associated with oxidative stress, altered glutathione metabolism and suppression of antibody-mediated immunity in 5-week old common loon chicks dosed with 0.4 μg/g Hg (Kenow et al., 2008, 2007). Similarly, a variety

Table 3
Arithmetic mean, standard deviation, geometric mean, and range of total Hg concentrations in breast feathers of bald eagle nestlings sampled throughout North America.

Year	Region	Habitat type	Breast feather Hg (μg/g fw)[a]					Study
			n[b]	Mean	SD	GeoMean	Range	
Marine-influenced (estuarine and marine)								
2003–15	*Penobscot River Watershed, Maine*	*Estuarine*	*8*	*7.30*	*4.3*	*6.4*	*3.0–16.1*	*This study*
2003–15	*Penobscot River Watershed, Maine*	*Marine*	*8*	*6.5*	*1.5*	*6.3*	*4.2–8.4*	*This study*
1991–92	Maine, statewide	Estuary	9	7.6	4.0	6.1	1.3–15.2	Welch, 1994[c]
2007–13	*Maine coast (reference for PRW)*	*Marine/Estuarine*	*39*	*4.8*	*3.5*	*4.0*	*1.2–19.3*	*This study*
1998–99	South Carolina	Marine/Inland	8–10	3.10	1.5		1.1–6.7	Jagoe et al., 2002[c]
1991–92	Maine	Marine	19–24	4.0	2.3	4.0	1.1–12.3	Welch, 1994[c]
Freshwater river								
2003–15	*Penobscot River Watershed, Maine*	*River*	*21*	*15.6*	*3.8*	*15.2*	*9.4–23.6*	*This study*
1991–92	Maine, statewide	River	5	13.3	2	13.5	9.5–17.2	Welch, 1994[c]
2006–08	Upper St. Croix Nat. Scenic Riverway, Wisconsin	River	19			6.8		Dykstra et al., 2010
2006–08	Lower St. Croix Nat. Scenic Riverway, Wisconsin	River	14			4.5		Dykstra et al., 2010
2006–08	Mississippi River, Wisconsin	River	51			3.1		Dykstra et al., 2010
2006–08	Montana (southwestern)	River	16[d]			3.0		Harmata, 2011
Lake								
1985–89	Voyageurs National Park, Minnesota	Lake	8			20.0	5.2–27.0	Bowerman et al., 1994
2003–15	*Penobscot River Watershed, Maine*	*Lake*	*46*	*21.1*	*8.5*	*19.6*	*8.8–46.9*	*This study*
1991–92	Maine, statewide	Lake	12–15	20.2	7.6	18.8	8.0–36.7	Welch, 1994[c]
2000–02	British Columbia, Pinchi Lake (Hg mine)	Lake	12	18.0	6.1		10.0–28.0	Weech et al., 2006
1985–89	Great Lakes, Interior Lower Peninsula, Michigan	Lake	28			8.8	4.6–14.0	Bowerman et al., 1994
1985–89	Great Lakes, Lake Superior, Wisconsin/Michigan	Lake	19			8.7	2.7–18.0	Bowerman et al., 1994
1985–89	Great Lakes, Interior Upper Peninsula, Michigan	Lake	44			8.1	3.5–16.0	Bowerman et al., 1994
1985–89	Great Lakes, Lakes Michigan and Huron, Michigan	Lake	10			8.0	4.1–14.0	Bowerman et al., 1994
2000–02	British Columbia, (central), multiple sites	Lake	31	7.1	1.8		3.6–12.5	Weech et al., 2006[e]
2005–10	Voyageurs National Park, Minnesota, Rainy Lake	Lake	62			6.1		Pittman et al., 2011
2005–10	Voyageurs National Park, Minnesota, Namakan Lake	Lake	53			5.6		Pittman et al., 2011
1991–93	Florida (central)	Lake (mesotrophic)	28[d]	4.7			0.8–10.7	Wood et al., 1996
2006–08	Lake Superior, Wisconsin	Lake	29			3.9		Dykstra et al., 2010
1991–93	Florida (central)	Lake (eutrophic)	32[d]	3.5			0.9–14.3	Wood et al., 1996
1985–89	Great Lakes, Lake Erie, Ohio/Michigan	Lake	6			3.7	1.5–7.4	Bowerman et al., 1994
2005–10	Voyageurs National Park, Minnesota, Kabetogama	Lake	63			1.1		Pittman et al., 2011

[a] Table entries sorted from largest to smallest mean value within habitat groupings. For the purposes of study comparisons, we assume fresh weight (fw) is equivalent to dry weight (dw) (R. Taylor, TERL, pers. comm., mean % feather moisture < 1%; det. limit = 0.01%, n = 490).
[b] Sample size represents number of nesting territories unless noted otherwise.
[c] Annual means and standard deviations averaged. Sample size shows range of annual sample sizes.
[d] Sample size equals number of individual nestlings sampled.
[e] Mean represents the average Hg concentration of four multi-territory lakes used as references to Pinchi Lake in Weech et al. (2006). Standard deviations averaged.

of negative effects (neurological, immunological and histological; reduced appetite, growth, activity and willingness to hunt prey) were documented in juvenile great egrets (*Ardea albus*) dosed with 0.5 μg/g Hg (Bouton et al., 1999; Spalding et al., 2000a, 2000b). In our study, mean nestling blood Hg concentrations were ≥0.66 μg/g at 14% of nesting territories in freshwater river habitats, and 39% of territories sampled at lakes, while no nestlings sampled in marine or estuarine habitats exceeded this blood Hg concentration (Fig. 3). The risk for toxicological impacts of Hg may be most pronounced in recently fledged nestlings after the completion of feather development (Ackerman et al., 2011; Condon and Cristol, 2009; Fournier et al., 2002). Impacts of Hg could be particularly consequential during this period when fledgling eagles are developing critical survival skills and parental care diminishes (Bortolotti, 1984b; McCollough, 1986). Reduced survival of post-fledged nestlings, juveniles or non-breeding adults would not be detected in Maine using current survey monitoring protocols (Watts and Duerr, 2010), particularly given influences of steady regional population growth and immigration.

5.6. Comparing nestling bald eagle blood vs. feathers for biomonitoring value

Although toxicologists have used bald eagle nestling blood and feather samples to monitor spatial and temporal patterns of Hg exposure in the environment for decades (Bowerman et al., 1994; Frenzel, 1984; Krantz et al., 1970; Pittman et al., 2011), no studies have quantitatively assessed whether one tissue might be better for detecting differences among habitats or regions than the other. Findings in our study demonstrated feathers were more useful for detecting Hg exposure as a function of habitat types compared to blood. This pattern was evident in pairwise comparisons among habitat types in the PRW, in which mean Hg concentrations differed between freshwater river and lake habitats in breast feathers, but not in blood. Ingested MeHg is absorbed rapidly into the bloodstream (<8 h) in developing birds, and the half-life of Hg has been estimated to be 3d (Fournier et al., 2002). In contrast, Hg concentrations in breast feathers reflect Hg exposure over a longer period spanning from just prior to their emergence at approximately 4 weeks of age (Bortolotti, 1984b) to potentially the approximate time of sampling at 5–7 weeks of age. Blood is therefore more sensitive to short-term changes in Hg exposure, while breast feathers reflect an average of Hg exposure over the period of feather formation. Our findings therefore support the practice exercised by many researchers prioritizing bald eagle nestling breast feathers over blood for spatial and temporal Hg biomonitoring efforts (Bowerman et al., 1994; Dykstra et al., 2010; Pittman et al., 2011), particularly in studies comparing Hg among different habitats.

6. Conclusions

Mercury concentrations in the blood and feathers of bald eagle nestlings were higher in the zone impacted by the chlor-alkali plant when compared to the reference sites, supporting the conclusion that the facility contributed Hg to the food web relied upon by bald eagles in the lower Penobscot River and Penobscot Bay. While tissue Hg concentrations in bald eagle nestlings potentially exposed to Hg from HoltraChem were significantly higher than those found at reference sites, they were

significantly lower than those found in freshwater river and lake habitats unaffected by the chlor-alkali plant. This finding demonstrates the degree to which site-specific factors associated with habitat type have potential to both lower (i.e., through dilution and tidal flows) and enhance MeHg bioavailability to top predators. Bald eagles throughout interior Maine are commonly exposed to Hg at concentrations associated with reproductive impacts in common loons and neurological impacts in bald eagles.

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