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# Mercury concentrations in blood and feathers of nestling Bald Eagles in coastal and inland Virginia

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## Abstract

**Background:** Mercury (Hg) and methylmercury are widely considered significant issues for wildlife, and in particular, piscivorous birds due to their widespread availability and neurotoxic properties. Whereas a substantial number of studies of Hg contamination of Bald Eagles (*Haliaeetus leucocephalus*) have been conducted throughout the east coast of the United States, little has been done that directly addresses Hg contamination in Bald Eagles in Virginia, particularly the inland population.

**Methods:** We collected blood and feather samples from nestling Bald Eagles in the coastal plain, piedmont, and western regions of Virginia in an effort to determine which areas of the state were more likely to contain populations showing evidence of Hg toxicity. We analyzed the samples for total Hg using a Milestone DMA-80.

**Results:** Samples collected from individuals located in the coastal region exhibited low concentrations of Hg compared to those further inland located on freshwater rivers and reservoirs. Samples collected from the inland population exhibited levels in some areas that are approaching what may be considered to be sub-lethal to avian health (blood: mean 0.324 mg/kg, SE = 0.13, range = 0.06–0.97 mg/kg; feather: mean = 8.433 mg/kg, SE = 0.3, range = 3.811–21.14 mg/kg).

**Conclusions:** Even after accounting for known point-sources of Hg, the inland eagle population in Virginia is susceptible to concentrations of Hg that are significantly higher than their coastal counterparts. Moreover, several locations besides those currently known to be impacted by point-sources are exhibiting concentrations that are approaching a sub-lethal level.

**Keywords:** Bald Eagle, *Haliaeetus leucocephalus*, Mercury, Methyl-mercury, Virginia

## Background

It is well known that the neurotoxic properties of mercury (Hg) and methylmercury (MeHg) represent a substantial risk to wildlife, in particular, higher trophic level species (Scheuhammer 1987; Yates et al. 2004, 2005; Evers et al. 2005; Hopkins et al. 2013). Wolfe et al. (1998) suggested that the impacts of elevated Hg are likely more significant when the individuals are in the developmental stages of growth. Moreover, elemental Hg readily

methylates into its more toxic form given appropriate environmental conditions (sulfide and dissolved organic matter) (Miller et al. 2007), making it available to wildlife and humans (Thompson 1996; Evers et al. 2005; Kramar et al. 2005). The widespread transport of Hg via atmospheric processes, and subsequent atmospheric deposition, ensures that Hg will remain globally ubiquitous (Wiener et al. 2003). Much of the available Hg in the atmosphere is due, in large part, to a myriad of industrial sources such as chlor-alkali facilities (Maserti and Ferrara 1991), as well as the continued combustion of coal and other fossil fuels (Berlin et al. 2007). Due to extensive risks posed to humans and wildlife from the

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accumulation of Hg, every state within the continental US has fish consumption advisories already in place.

Research on Hg in Bald Eagles (*Haliaeetus leucocephalus*) has been conducted in numerous eastern states including South Carolina (Jagoe et al. 2002), Florida (Wood et al. 1996), Maine (Welch 1994), New York (DeSorbo et al. 2008, 2009), and the Great Lakes region of the US (Bowerman et al. 1994). Although the Bald Eagle has been studied extensively in the Coastal Plain of Virginia (Buehler 1990; Buehler et al. 1991; Watts et al. 2011), little research on Bald Eagles has been conducted in the inland regions, due in part to a lack of knowledge of the inland distribution. Wiemeyer et al. (1984) analyzed non-viable eggs from Bald Eagles in the Chesapeake Bay, and reported concentrations of Hg between 0.03 and 0.17 mg/kg, and Cristol et al. (2012) found that eagles residing near the Chesapeake Bay exhibited low concentrations of Hg in molted feathers. In contrast to the Chesapeake Bay, several inland areas in the state have been identified as hotspots for Hg exposure to other avian species, including the Shenandoah River (Jackson et al. 2011; Cristol et al. 2012; Hopkins et al. 2013) and the North Fork of the Holston River (Echols et al. 2009). Jackson et al. (2011) noted that forest songbirds were exhibiting elevated Hg concentrations over 130 km downstream of the known point source on the Shenandoah River, with Hg distributed in downstream habitat features such as flood plain areas.

Within the state of Virginia, the majority of the known eagle population ( $n=726$  occupied territories 2011) nests within the coastal plain associated with the Chesapeake Bay and the major tributaries that feed the bay (Watts et al. 2011). However, more than 25% of the total population ( $n>200$ ) nests in the Piedmont and Mountain regions (Kramar D., unpublished data). Prior to 2007, little information regarding the inland population was available. As elevated Hg in wildlife has been positively associated with freshwater environments, we expect that Hg concentrations in central and western Virginia eagles would exhibit significantly higher levels in blood and feather than those of the coastal plain. Moreover, as Hg accumulates in the body with age (Kamman et al. 2005), nestling eagles that are expressing levels at or above those considered sub-lethal are an important indicator of Hg in the immature and adult cohorts of the same eagle population.

Our objectives were to establish reference levels for Hg contaminants in Bald Eagles in coastal Virginia based on previous work (Cristol et al. 2012) and from collection and analysis of new tissue samples from nestling Bald Eagles. This allowed us to test our hypothesis that inland Hg concentrations were significantly higher than the Hg concentrations found in Bald Eagles in the coastal

population. We also evaluated the spatial variability of Hg across the inland population, and analyzed the relationship between blood Hg and feather Hg.

## Methods

### Study area and surveys

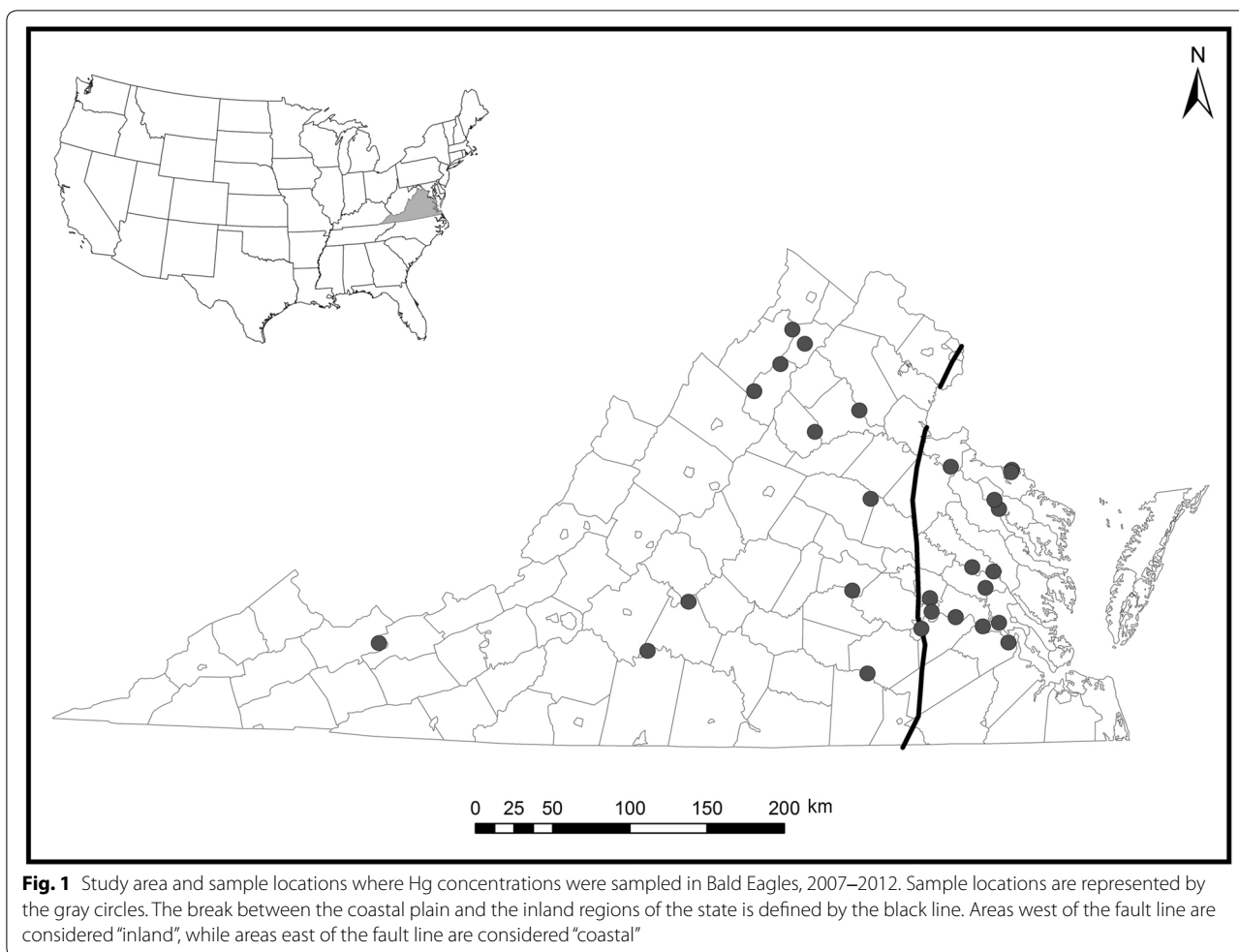
We conducted this throughout the state of Virginia between the spring of 2007 and the spring of 2012. The population of eagles in Virginia has rebounded from a low of approximately 80 pairs in the 1970s (Abbott 1978), to over 800 by 2012 for both the coastal and inland populations (Watts et al. 2011; Kramar D., unpublished data). We sampled 28 nests across the state, and 46 individuals (Fig. 1). In instances where there were multiple individuals per nest, we used the average Hg concentration between the individuals to represent the Hg concentration per nest. We also checked to see if using either the minimum or maximum values had any effect on the resulting models. Coastal nests were defined as those that fell within the coastal plain (east of the fault line), and inland nests were defined as those that fell within the Piedmont and Mountain physiographic regions (west of the fault line). Nests were randomly selected and assessed for climbability based on the condition of the tree, and the age of the individuals. In a small number of cases, selected nests could not be climbed due to lack of approval from the property owner. In these cases, we selected another nest.

### Blood and feather collection

We collected samples from 4 to 7 week old eaglets by climbing to the nests and lowering them to the ground in a protective bag. We collected blood samples from the brachial vein in the wing using 21 gauge butterfly needles and 4 cc lithium heparinized vacutainers. Each vacutainer was labeled with a unique nest identification number and the federal band number, and the blood was packed on ice and frozen within 4 h of collection. We collected two to three contour feathers from the breast of each individual. The samples were placed in a brown envelope, sealed, and labeled with the nest identification number and federal band number. All activities were conducted under the appropriate state and federal permits.

### Blood and feather analysis

Samples were analyzed using a Milestone DMA-80 Direct Mercury Analyzer (DMA) at the Sawyer Environmental Research Center, located at the University of Maine, Orono. Analysis of samples was conducted after calibrating the DMA using a certified standard Hg solution (SPEX CertiPrep) (Cell 1:  $R^2=1.0000$ , Cell 2:  $R^2=0.9998$ ). All calibration and sampling consisted of measuring both the SPEX and the samples



to the nearest 0.0001 g using a Metler Toledo digital scale, Model AT201). Quality assurance measures included the use of standard reference materials. In addition to the standard reference materials, two system and method blanks, one 10-ng prepared Spex sample, one 60-ng prepared Spex sample, one replicate, and one matrix spike were run as quality assurance per every ten biological samples. Feathers were mechanically washed following Ackerman et al. (2007) in a 1% Alconox solution, rinsed 6 times in deionized water, and dried for 24–48 h. The recovery of standard reference materials was near 100% and well within the accepted range of 90–110%. QC verification was 105.6% and 103.1% during calibration. Similarly, replicate samples were well within accepted parameters. Spiked samples were based on the addition of 0.05 g of 10 ng SPEX. Once prepared, samples were weighed out and placed in quartz or nickel boats for analysis (depending on the matrix type: quartz = blood, nickel = feather). No more

than 20 samples were analyzed at any one time, with QC procedures conducted following every ten samples. Analysis generally followed US Environmental Protection Agency Method 7473 (USEPA 2000).

#### Statistical analysis

We conducted a power analysis both prior to collection of samples and post sampling, to insure that the sample size was adequate to make inferences regarding the population. Given a standard deviation across all samples of 0.205, and 28 samples (individual nests), the power to determine differences between the coastal and inland populations at  $\alpha = 0.25$  was 0.874.

We initially log-transformed the Hg scores and applied the Shapiro Wilks Goodness of Fit test to determine whether they met normality requirements. Because the log-transformed data did not approximate a normal distribution, we compared the Hg in coastal and inland populations using the non-parametric

Wilcoxon test. To determine the effect of point contaminant sources on the Shenandoah River, we ran a second Wilcoxon test while withholding the data from the Shenandoah River.

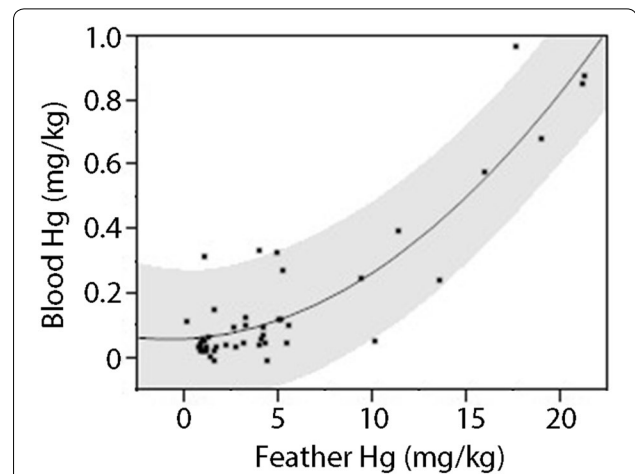
To test for a relationship between blood Hg and feather Hg, we developed a predictive model of the function. We initially fit a linear regression model and evaluated the fit by analyzing the residual distribution. Due to the non-linear nature of our sample distribution we utilized a non-linear quadratic regression to appropriately model the relationship.

## Results

The mean blood Hg concentration across Virginia eagles was  $\bar{x}=0.180$  mg/kg, SE=0.036. The mean concentration for feather Hg was  $\bar{x}=5.024$  mg/kg, SE=0.842. The lowest blood Hg concentrations sampled (0.012 mg/kg, SE=0.012) were located in birds from the coastal plain of Virginia, specifically on the Pamunkey River. The highest concentrations were sampled in birds located inland on the Shenandoah River, in particular on the South Fork of the Shenandoah River (0.974 mg/kg, SE=0.065).

Blood Hg concentrations in the coastal plain ( $\bar{x}=0.06$  mg/kg, SE=0.012 mg/kg, range=0.01–0.15 mg/kg) were significantly less ( $Z=4.676$ ,  $p<0.0001$ ,  $\chi^2=21.96$ ,  $p<0.0001$ ) than blood Hg concentrations in inland nests ( $\bar{x}=0.324$  mg/kg, SE=0.065 mg/kg, range=0.06–0.97 mg/kg). Feather Hg concentrations in the coastal plain ( $\bar{x}=2.16$  mg/kg, SE=0.420 mg/kg, range=0.62–9.998 mg/kg) were significantly less ( $Z=4.168$ ,  $p<0.0001$ ,  $\chi^2=17.465$ ,  $p\leq 0.0001$ ) than feather Hg concentrations in inland nests ( $\bar{x}=8.43$  mg/kg, SE=1.47, range=3.81–21.14 mg/kg). After removing the Shenandoah River from the analysis, we still found that the coastal blood Hg concentrations were significantly less ( $Z=4.0$ ,  $p<0.0001$ ,  $\chi^2=16.08$ ,  $p<0.0001$ ) than blood Hg concentrations in the inland nests. Likewise, after removing the Shenandoah River from the analysis, feather Hg concentrations from the coastal plain were significantly less ( $Z=3.38$ ,  $p=0.0007$ ,  $\chi^2=11.52$ ,  $p=0.0007$ ) than feather Hg concentrations from the inland nests.

The quadratic regression model provided the best overall fit to our data (Fig. 2). This cumulative function (Fig. 2) supported the prediction that Hg in feathers accumulated over time, representing total body burden, while Hg in blood represented recent dietary uptake. In that respect, the relationship between feather and blood



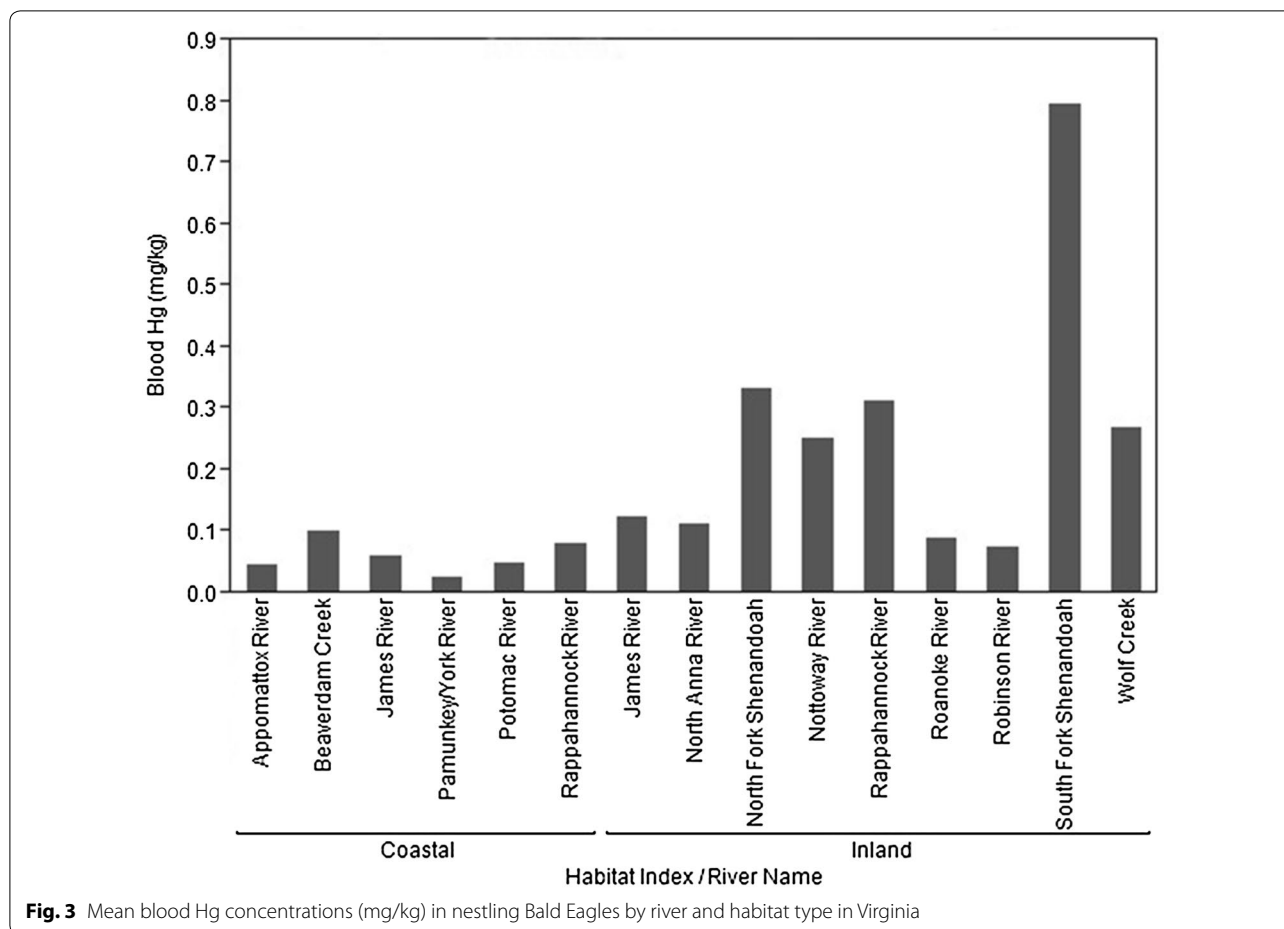
**Fig. 2** Quadratic fit of the relationship between Hg in feathers and blood of nestling Bald Eagles. (Blood\_Hg = 0.0185943 + 0.02095 · Feather\_Hg + 0.0017723 · (Feather\_Hg – 5.02407)<sup>2</sup>. The shaded area represents the confidence fit

Hg, in this study, is a cumulative function. The analysis of variance table indicated a sufficient model fit ( $F=12.97$ ,  $df=1$ ,  $SS=0.133$ ,  $p=0.001$ ) that represents 83.4% of the variance of blood Hg concentrations.

## Discussion

While we found that the coastal population of nestling Bald Eagles in Virginia had Hg concentrations that are not of a level currently of concern, the inland population has Hg burdens that are at significantly higher concentrations than previously expected. These findings support Cristol et al. (2012) who also documented low levels of Hg in Bald Eagles in coastal Virginia. To establish a threshold for what may be considered sub-lethal concentrations of Hg, we used a blood Hg concentration of 0.70 mg/kg (Desorbo and Evers 2007). Whereas Bald Eagles along several inland rivers exhibited blood Hg levels approaching that threshold, only the Shenandoah River exceeded it (Fig. 3). No eagles along rivers in coastal Virginia approached this threshold. Specifically, rivers where eagles exhibited elevated concentrations include the inland portions of the Rappahannock River, Nottoway River, James River, and Wolf Creek. The higher concentrations of feather Hg found in this study, not surprisingly, follow patterns similar to that of the blood Hg concentrations (Fig. 4).

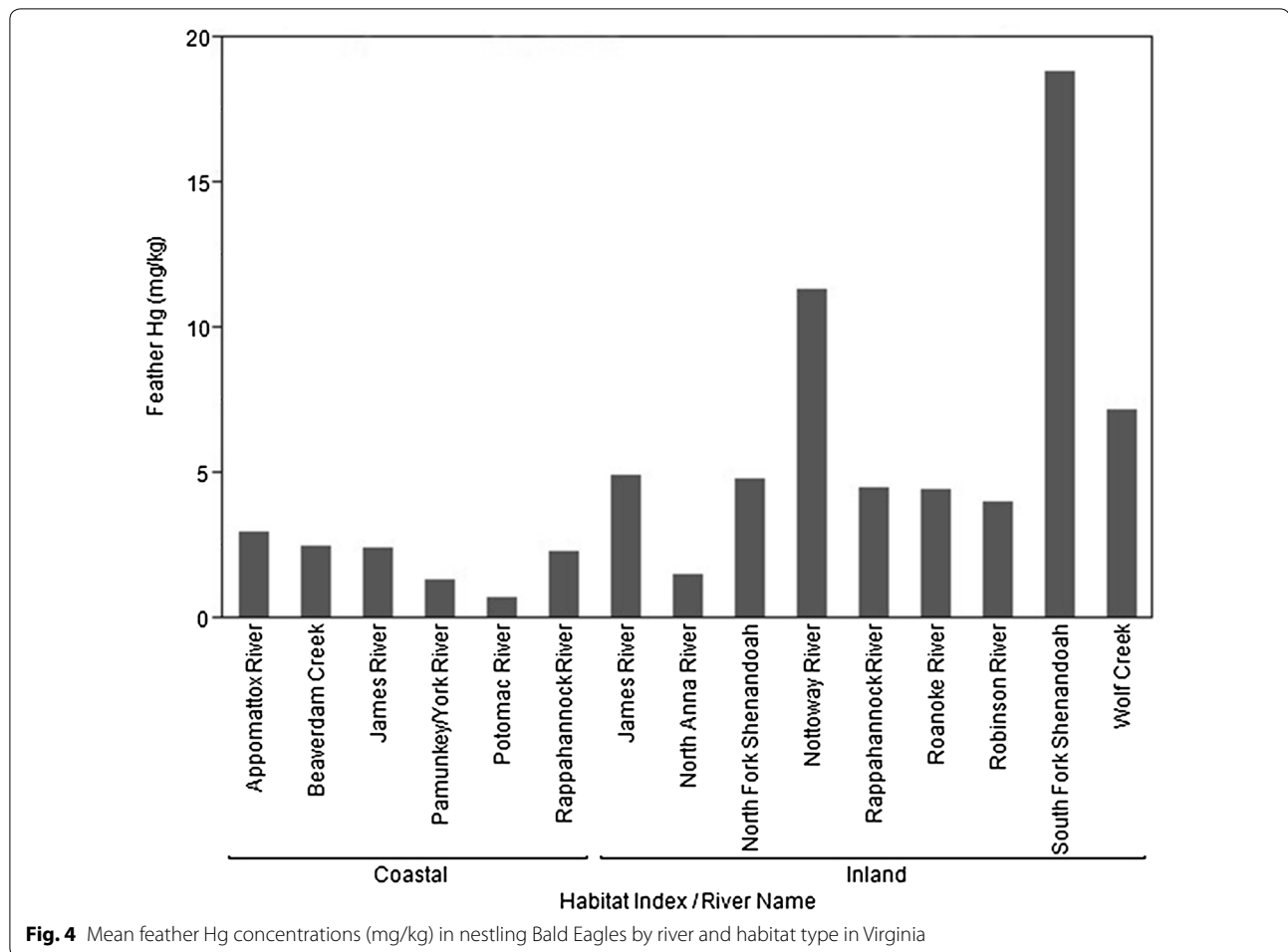
Given that Hg is sequestered rapidly during feather growth, it is not unreasonable to expect that once



feather development is complete, levels of blood Hg would increase. Condon and Cristol (2009) suggest that the most likely period for Hg to approach sub-lethal levels is immediately following fledging, when feathers are no longer sequestering Hg from the blood. Thus an individual at 4 weeks of age, which is approaching a 0.70 mg/kg threshold, would likely exceed that threshold once the feathers have completely developed. This relationship between blood Hg and feather growth in Bald Eagles identifies the need for additional sampling of adult Bald Eagles in inland Virginia. Moreover, additional research should be conducted on the North Fork of the Shenandoah River as Hg concentrations here were also approaching the 0.70 mg/kg threshold.

**Conclusions**

Inland eagle populations within Virginia are exposed to a greater amount of Hg than their coastal counterparts. This variation in Hg concentrations is likely due to a number of different factors. First, Hg methylates more readily in freshwater environments, and as such, that Hg would be available to wildlife that reside there. Second, we speculate that the elevated concentrations of Hg in inland eagle populations are also influenced by the variation in potential prey between the freshwater and saline environments. Because of these factors, the freshwater environment of the Piedmont and Mountain regions warrants continued monitoring to understand the long-term implications of Hg in these areas. In particular, additional work along the Nottoway River, Holsten River,



Shenandoah River, Rappahannock River, and Wolf Creek should be conducted.

From a management perspective, additional efforts to facilitate remediation and further understand the full distribution and potential implications of Hg within the inland portions of the state should be considered. These efforts should include research into potential point sources on the above mentioned rivers, as well as further research into the Hg concentrations in adult Bald Eagles. Lastly, given the Hg concentrations found on the rivers sampled in this research, additional efforts should be focused on sampling rivers that were not represented such as the Powell River, the Holston River, and the New River.

#### Authors' contributions

DEK collected and analyzed the data associated with this work. BC aided in editorial suggestions, geospatial analysis, and presentation of material. SP assisted in analysis, editorial suggestions, and geospatial analysis. JC assisted in editorial comments and statistical analysis. All authors read and approved the final manuscript.

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#### Competing interests

The authors declare they have no competing interests.

#### Availability of data and materials

The datasets generated during and/or analyzed during this study are available from the corresponding author on reasonable request.

#### Consent for publication

Not applicable.



**Ethics approval and consent to participate**

IACUC approval was secured through Virginia Polytechnic Institute and State University for the duration of the project.

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