



RESEARCH ARTICLE

## Feather mercury increases with feeding at higher trophic levels in two species of migrant raptors, Merlin (*Falco columbarius*) and Sharp-shinned Hawk (*Accipiter striatus*)

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### ABSTRACT

Mercury (Hg) is a toxic heavy metal that, when methylated to form methylmercury (MeHg), bioaccumulates in exposed animals and biomagnifies through food webs. The purpose of this study was to assess Hg concentrations in raptors migrating through the upper midwestern USA. From 2009 to 2012, 966 raptors of 11 species were captured at Hawk Ridge, Duluth, Minnesota, USA. Breast feathers were sampled to determine the concentration of total Hg. Mean Hg concentrations ranged from 0.11 to 3.46  $\mu\text{g g}^{-1}$  fresh weight across species and were generally higher in raptors that feed on birds in comparison with those that feed on mammals. To evaluate the effect of dietary sources on Hg biomagnification, carbon and nitrogen stable isotope ratios were measured in feathers of the 2 species with the highest Hg concentrations, Merlin (*Falco columbarius*) and Sharp-shinned Hawk (*Accipiter striatus*). Measured  $\delta^{13}\text{C}$  values were similar in both species and indicated a primarily terrestrial-derived diet, whereas  $\delta^{15}\text{N}$  values suggested that individual Merlin and Sharp-shinned Hawk feeding at higher trophic levels accumulated higher concentrations of Hg. The risk to birds associated with measured levels of feather Hg was evaluated by calculating blood-equivalent values using an established algorithm. Predicted blood values were then compared to heuristic risk categories synthesized across avian orders. This analysis suggested that while some Merlin and Sharp-shinned Hawk were at moderate risk to adverse effects of MeHg, most of the sampled birds were at negligible or low risk.

**Keywords:** bioaccumulation, biomagnification, falcons, hawks, methylmercury, owls, stable isotopes

### El mercurio de las plumas aumenta con los alimentos de niveles tróficos más altos en dos especies de rapaces migratorias, *Falco columbarius* y *Accipiter striatus*

### RESUMEN

El mercurio (Hg) es un metal pesado tóxico que, cuando se metila para formar metilmercurio (MeHg), se acumula en los tejidos de los animales expuestos y se bio-magnifica a través de las redes tróficas. El propósito de este estudio fue evaluar las concentraciones de Hg en rapaces que migran a través de la parte alta del medio oeste de Estados Unidos. De 2009 a 2012, capturamos 966 rapaces de 11 especies en Hawk Ridge, Duluth, Minnesota, EEUU. Tomamos muestras de plumas del pecho para determinar la concentración de Hg total. Las concentraciones medias de Hg fluctuaron entre 0.11 y 3.46  $\mu\text{g g}^{-1}$  de peso fresco para las distintas especies y fueron generalmente más altas en las rapaces que se alimentan de aves en comparación con aquellas que se alimentan de mamíferos. Para evaluar el efecto de las fuentes de alimento sobre la bio-magnificación de Hg, se midieron los cocientes de isótopos estables de carbono y nitrógeno en las plumas de las dos especies con las concentraciones más altas de Hg, *Falco columbarius* y *Accipiter striatus*. Los valores medidos de  $\delta^{13}\text{C}$  fueron similares en ambas especies e indicaron una dieta derivada principalmente del medio terrestre, mientras que los valores de  $\delta^{15}\text{N}$  sugirieron que los individuos de *F. columbarius* y *A. striatus* que se alimentan a niveles tróficos más altos acumularon concentraciones más altas de Hg. El riesgo para las aves asociado con los niveles medidos de Hg en las plumas fue evaluado mediante el cálculo de valores equivalentes en sangre usando un algoritmo establecido. Los valores predichos en sangre fueron luego comparados con categorías de riesgo heurístico sintetizadas a través de los órdenes de aves. Este análisis sugirió que mientras algunos individuos de *F. columbarius* y *A. striatus* tuvieron un riesgo moderado a los efectos adversos de MeHg, la mayoría de las aves muestreadas tuvieron un riesgo insignificante o bajo.

**Palabras clave:** bio-acumulación, bio-magnificación, búhos, halcones, isótopos estables, metilmercurio

## INTRODUCTION

For decades, researchers have used standardized raptor count data to assess population trends of North American raptors. These data have proven useful for identifying populations in decline (Farmer et al. 2007). A variety of factors have been implicated in broad-scale population declines of raptors, including environmental contaminants such as pesticides (e.g., DDT; Grier 1982, Falk et al. 2006), industrial chemicals (e.g., PCBs and dioxins; Bowerman et al. 1995), and pharmaceuticals (e.g., diclofenac; Green et al. 2004). In such cases, raptors may act as indicator species (Niemi and McDonald 2004) for potential contaminant impacts on other species, including humans (Bowerman et al. 2002).

Mercury (Hg) is another contaminant of concern for raptors (Albers et al. 2007). While occurring naturally in the environment, human activities such as chlor-alkali production, combustion of fossil fuels, and gold mining have increased the availability of Hg to aquatic and terrestrial wildlife (Wiener et al. 2003, Driscoll et al. 2007, 2013; Evers et al. 2007). Of special concern is the methylated form of Hg (MeHg), which is formed from inorganic  $\text{Hg}^{2+}$  by sulfate-reducing bacteria (Compeau and Bartha 1985, Ullrich et al. 2001). In addition to its high toxicity, MeHg tends to accumulate in tissues over time (i.e. bioaccumulation; Haney and Lipsey 1973, Scheuhammer et al. 2011). Dietary uptake of MeHg is highly efficient and can result in increasing MeHg concentrations at successively higher trophic levels (i.e. biomagnification; Jaeger et al. 2009).

Biogeochemical conditions present in aquatic ecosystems are generally more conducive to Hg methylation than those in terrestrial ecosystems (Compeau and Bartha 1985, Ullrich et al. 2001). As a result, much of the research to date on Hg in birds has been focused on piscivorous birds such as the Common Loon (*Gavia immer*; Meyer et al. 1995, Evers et al. 1998a, 2008; Scheuhammer et al. 1998), Bald Eagle (*Haliaeetus leucocephalus*; Bowerman et al. 2002, Rutkiewicz et al. 2011, DeSorbo et al. 2018), and Osprey (*Pandion haliaetus*; Anderson et al. 2008, Rumbold et al. 2017). More limited data exist for species associated with terrestrial ecosystems such as the American Kestrel (*Falco sparverius*; Albers et al. 2007, Fallacara et al. 2011), Peregrine Falcon (*F. peregrinus*; Mora et al. 2002, Barnes and Gerstenberger 2015), Sharp-shinned Hawk (*Accipiter striatus*; Wood et al. 1996), Golden Eagle (*Aquila chrysaetos*; Langner et al. 2015), Red-tailed Hawk (*Buteo jamaicensis*; Bourbour et al. 2019), Red-shouldered Hawk (*B. lineatus*; Bourbour et al. 2019), and Cooper's Hawk (*Accipiter cooperii*; Bourbour et al. 2019).

Birds eliminate MeHg by several routes including loss in urine-feces, elimination through hepatic demethylation (Thompson and Furness 1989, Scheuhammer et al. 1998), and deposition into eggs (Becker 1992, Lewis et al. 1993,

Heinz and Hoffman 2004, Ackerman et al. 2007, French et al. 2010). For most birds, however, the primary route of MeHg elimination is deposition into feathers (Honda et al. 1986, Braune and Gaskin 1987, Fournier et al. 2002, Nichols et al. 2010, Whitney and Cristol 2017a). Feathers reflect circulating levels of MeHg at the time they were formed. Specific feathers can represent different, discrete periods of exposure throughout an individual's life history because molting patterns are often documented and predictable (Ramos et al. 2009). Over 95% of Hg in feathers exists as MeHg (Thompson and Furness 1989, Thompson et al. 1990, Evers et al. 2005). Measured total Hg in feathers may therefore be used as an index of MeHg exposure (Evers et al. 2005, 2011; Rimmer et al. 2005).

Stable isotope analysis of carbon and nitrogen in feathers can be used to evaluate relative trophic position and to trace MeHg sources in the diet (Atwell et al. 1998, Tavares et al. 2009, Lavoie et al. 2013, Carravieri et al. 2014, Øverjordet et al. 2015). The isotopic composition of tissues reflects the diet during the period of tissue growth and maintenance (Hobson and Clark 1992, Bearhop et al. 2002). Thus, the ratio of  $^{15}\text{N}:^{14}\text{N}$  in feathers (the  $\delta^{15}\text{N}$  value) can be used to determine the trophic position relative to a baseline and of one individual or species relative to another (Thompson and Furness 1995, Bearhop et al. 2002). On average, the trophic discrimination in birds (the increase in  $^{15}\text{N}$  relative to  $^{14}\text{N}$  between feathers and diet) is  $3.8 \pm 0.3\%$  (mean  $\pm$  SE; Caut et al. 2009). In contrast,  $\delta^{13}\text{C}$  values of an organism's tissues tend to more closely resemble those of their diet (average trophic discrimination of  $2.2 \pm 0.4\%$ ; Caut et al. 2009). However, the  $\delta^{13}\text{C}$  value may be useful to trace dietary sources because  $\delta^{13}\text{C}$  values vary among primary producers and ecosystem types (Walker et al. 2015). In particular,  $\delta^{13}\text{C}$  values for phytoplankton in lake food webs are generally lower (less than  $-30\%$ ) than values associated with plants in terrestrial food webs (typically  $-30\%$  to  $-26\%$ ; Peterson and Fry 1987). The existence of a lower  $\delta^{13}\text{C}$  value in feathers may therefore provide evidence for consumption of food associated with freshwater ecosystems (Hebert et al. 2009), recognizing that littoral and wetland habitat food webs often have  $\delta^{13}\text{C}$  values similar to or more  $^{13}\text{C}$ -enriched than terrestrial food webs (Gurney et al. 2017).

The primary purpose of this study was to assess feather Hg concentrations in migrant raptors using the Central Flyway of North America in autumn. In addition, stable isotopes were measured in 2 species, Merlin (*Falco columbarius*) and Sharp-shinned Hawk, to compare their relative trophic position and to characterize likely dietary sources (aquatic vs. terrestrial food sources). We hypothesized that after hatch year (AHY), or adult birds, would have higher feather Hg concentrations than hatch year (HY), or first-year birds, because AHY birds have a longer exposure history and more limited Hg depuration options compared

to HY birds, which lessen their overall Hg burden through mass dilution and extensive feather growth during nestling development. We also predicted that birds feeding at a higher trophic level (as indicated by higher  $\delta^{15}\text{N}$ ) or consuming prey from an aquatic-based food web (as indicated by lower  $\delta^{13}\text{C}$ ) would have higher concentrations of feather Hg than those feeding at a lower trophic level or consuming prey from a terrestrial-based food web.

## METHODS

### Study Area

All samples were collected between 2009 and 2012 during fall migration (September through November) from the Hawk Ridge Bird Observatory banding station at Duluth, Minnesota, USA (46.85°N, -92.03°W), located near the westernmost point of Lake Superior. Situated at ~300 m above sea level, Hawk Ridge is positioned on basalt rock that extends along the north shore of the lake. This unique location concentrates large numbers of birds of prey, and on average, the banding station bands over 2,600 raptors each fall (Evans et al. 2012). Limited band recovery data suggest that diurnal raptors migrating past Duluth likely originate from breeding grounds in western Canada and northern Minnesota (Evans et al. 2012).

### Raptor Sampling

Birds were captured using mist, bow, and dho gaza nets (Evans et al. 2012; Table 1). Captured birds were identified, aged, and sexed using methods described by Pyle (2008). Two to four breast feathers were plucked from each sampled bird, inspected for external debris, and placed into envelopes. Feathers were chosen because they represent a reliable index of Hg exposure during the time of feather growth (Bearhop et al. 2000, Kenow et al. 2007, Condon and Cristol 2009) and because they can be sampled non-invasively and easily preserved at room temperature for long periods.

### Mercury Analysis

One feather per individual was analyzed for total Hg concentration by thermal decomposition spectrophotometry (EPA method 7473) using a DMA-80 Direct Mercury Analyzer (Milestone) at the BRI Toxicology Lab at Biodiversity Research Institute in Portland, Maine, following methods described by Evers et al. (2005). Feathers were prepared for analysis using a protocol employed by U.S. Fish and Wildlife Service contract laboratories (e.g., Texas A&M University; R. Taylor, personal communication). Only feathers with no visual evidence of external debris were analyzed. Feathers were not washed, as this protocol does not recommend washing unless external contamination is of concern. Quality control methods including the use of analytical blanks, sample replicates, and certified reference materials DOLT-4, DORM-3, and DORM-4, were employed to evaluate analytical precision and accuracy. Total Hg concentrations in all feathers were above the method detection limit ( $0.001 \mu\text{g g}^{-1}$ ). Measured Hg concentrations in Certified Reference Materials (CRMs, National Research Council, Canada, and Joint Research Centre, European Union) incorporated into each sample run averaged 100% (DOLT-4), 103.7% (DORM-3), and 97.9% (DORM-4) of published values. Feather Hg concentrations are presented in  $\mu\text{g g}^{-1}$  on a fresh weight (fw) basis. Previous work has shown that Hg concentrations can vary among feathers in different parts of the plumage of individual birds (Furness et al. 1986, Braune and Gaskin 1987, Peterson et al. 2019). Although we did not sample multiple feather tracts, we controlled for variation among feathers within birds and standardized comparisons across birds by limiting our sampling to only breast feathers.

We also analyzed limited duplicates (e.g., a separate breast feather from the same individual) to verify consistency in Hg concentrations among breast feathers within the same individuals. For duplicates we calculated percent relative difference and Pearson's correlation coefficient. Because HY birds were known to have grown all feathers

**TABLE 1.** Number of individuals sampled. All samples are from Hawk Ridge, Duluth, Minnesota, USA, and were collected from 2009 to 2012. Sample sizes are given for individual age and sex classes. Age is either hatch year (HY), after hatch year (AHY), or unknown (U); sex is either male (M), female (F), or unknown (U).

Common name	Scientific name	Total	HY	AHY	U	Male	Female	U
Northern Harrier	<i>Circus hudsonius</i>	30	25	5	0	17	13	0
Sharp-shinned Hawk	<i>Accipiter striatus</i>	392	197	195	0	179	213	0
Cooper's Hawk	<i>Accipiter cooperii</i>	11	4	7	0	7	4	0
Northern Goshawk	<i>Accipiter gentilis</i>	222	196	26	0	152	70	0
Red-shouldered Hawk	<i>Buteo lineatus</i>	1	1	0	0	0	0	1
Broad-winged Hawk	<i>Buteo platypterus</i>	5	5	0	0	0	0	5
Swainson's Hawk	<i>Buteo swainsoni</i>	1	1	0	0	0	0	1
Long-eared Owl	<i>Asio otus</i>	88	15	68	5	7	7	74
American Kestrel	<i>Falco sparverius</i>	71	53	5	13	37	34	0
Merlin	<i>Falco columbarius</i>	137	117	20	0	77	60	0
Peregrine Falcon	<i>Falco peregrinus</i>	8	8	0	0	5	3	0

within a recent and well-defined timeframe (i.e. nestling development), we also analyzed duplicates and calculated the same statistics within HY and AHY age class groups. The fw of a feather is nearly equivalent to dry weight (dw; R. Taylor, TERL, Texas A&M University; mean % feather moisture <1%,  $n = 490$ , reported in DeSorbo et al. 2018). We therefore considered fw = dw for the purposes of literature comparisons.

### Stable Isotope Analysis

Ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotopes in feathers were measured for the 2 species of raptors with the highest mean Hg concentrations: Merlin and Sharp-shinned Hawk. This was done to evaluate the potential relationship between feather Hg and trophic status (as measured by  $\delta^{15}\text{N}$ ), and aquatic vs. terrestrial carbon source in the raptor diet (as measured by  $\delta^{13}\text{C}$ ). Samples from 20 individuals within each age and sex class were selected for stable isotope analysis, with 10 feathers each from the lowest and highest Hg results for each class. Each feather sample was cleaned with a 2:1 chloroform:methanol solution (Hobson 1999), placed in a pre-combusted scintillation vial, and dried at 50°C for at least 24 hr. After drying, the sample was minced and 0.7  $\mu\text{g}$  analyzed with a Costec 4010 EA and Thermo Delta Plus XP isotope ratio mass spectrometer. Stable isotope ratios are reported in standard  $\delta$  notation, wherein Vienna Pee Dee Belemnite and air are standards for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively. Analytical error, calculated as the mean standard deviation of replicate reference material, was <0.1‰ for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

### Statistical Analyses

Measured feather Hg concentrations were natural log-transformed, and geometric mean concentrations ( $\pm$  SE) were calculated for all 11 species. For a subset of 6 species with greater sample sizes ( $n \geq 20$ ), analysis of variance was used to compare ln-transformed Hg concentrations, ln(feather Hg), by sex within species, age within species, and their interaction (cohort = all combinations of age and sex) within species.

Additional statistical analyses were performed for Merlin and Sharp-shinned Hawk, our 2 largest samples. A set of 18 general linear models was developed to compare ln(feather Hg) to the year in which birds were captured, ordinal day on which birds were captured, bird age class, sex, and all bivariate interaction terms (Table 2). Year was modeled as a quantitative covariate to test whether feather Hg concentrations were changing over the course of the 4 years of study. Date was also modeled as a quantitative covariate to explore seasonal variation in feather Hg. Age and sex were modeled as factors, each with 2 levels. Variable importance was calculated based on Akaike's information criterion, corrected for small sample size ( $\text{AIC}_c$ ),

as the sum of  $\text{AIC}_c$  weights ( $w_i$ ) for each model in which the variable occurred (Burnham and Anderson 2002). A list of all models considered for Merlin and Sharp-shinned Hawk is provided (Table 2).

Correlations between ln(feather Hg) and  $\delta^{13}\text{C}$ , and between ln(feather Hg) and  $\delta^{15}\text{N}$  by sex, age, and cohort within each species were evaluated using Pearson's correlation coefficient. All analyses were conducted in R 2.14.0 (R Development Core Team 2013).

## RESULTS

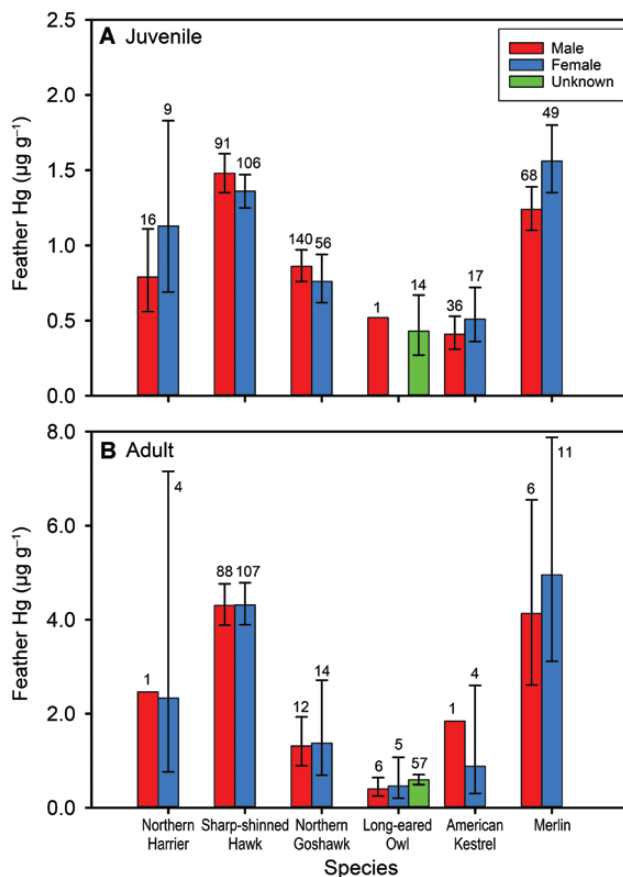
Mercury (as total Hg) was detected in all 966 feathers from 11 species (Appendix Table 10). We had sufficient data for ANOVA on age, sex, and age\*sex for 6 species (Table 3). Geometric mean feather Hg concentrations were significantly lower in HY than AHY individuals in all but one species, Long-eared Owl (*Asio otus*; Figure 1). A main effect of sex ( $F > M$ ) was observed only for Merlin and combined age and sex categories (an age\*sex interaction) were significant for 4 species (Table 3). Regardless of the interaction between age and sex, AHY Merlin and Sharp-shinned Hawk had the highest geometric mean ( $\pm$  geometric standard deviation) Hg concentrations, 4.56  $\mu\text{g g}^{-1}$  fw (2.02) and 4.30  $\mu\text{g g}^{-1}$  fw (1.68), respectively (Figure 1). Similarly, geometric mean Hg concentrations in HY Merlin and Sharp-shinned Hawk were higher than those in HY birds of other species, 1.37  $\mu\text{g g}^{-1}$  fw (1.66) and 1.41  $\mu\text{g g}^{-1}$  fw (1.53), respectively; Figure 1).

**TABLE 2.** Model list and R syntax for 18 linear models for Sharp-shinned Hawk and Merlin banded at Hawk Ridge, Duluth, Minnesota, USA, 2009–2012. Date is the ordinal day from 1 to 365; year is a factor that takes values 2009, 2010, 2011, and 2012; sex is either male or female; age is either hatch year (HY) or after hatch year (AHY). lnHg is natural log of total feather Hg concentration. Models with interaction terms always include the associated main effects.

Model name	Model statement in R
Age	lm1 <- lm(lnHg ~ Age)
Sex	lm2 <- lm(lnHg ~ Sex)
Year	lm3 <- lm(lnHg ~ Year)
Date	lm4 <- lm(lnHg ~ Date)
Age + Sex	lm5 <- lm(lnHg ~ Age + Sex)
Age + Year	lm6 <- lm(lnHg ~ Age + Year)
Age + Date	lm7 <- lm(lnHg ~ Age + Date)
Sex + Year	lm8 <- lm(lnHg ~ Sex + Year)
Sex + Date	lm9 <- lm(lnHg ~ Sex + Date)
Year + Date	lm10 <- lm(lnHg ~ Year + Date)
Sex*Age	lm11 <- lm(lnHg ~ Date + Sex * Age)
Date*Age	lm12 <- lm(lnHg ~ Date * Age)
Year + Sex*Age	lm13 <- lm(lnHg ~ Year + Sex * Age)
Sex*Age	lm14 <- lm(lnHg ~ Sex * Age)
Date*Year + Sex*Age	lm15 <- lm(lnHg ~ Date * Year + Sex * Age)
Date*Year	lm16 <- lm(lnHg ~ Date * Year)
Date*Sex	lm17 <- lm(lnHg ~ Date * Sex)
Null	lm18 <- lm(lnHg ~ 1)

**TABLE 3.** Analysis of variance results for natural log-transformed Hg concentrations [ln(Hg)] in feathers by sex, age, and all sex and age combinations (cohort). All samples were collected from Hawk Ridge, Duluth, Minnesota, USA, from 2009 to 2012. Bold values indicate statistical significance of  $P \leq 0.05$ . For age and sex,  $df = 1$ . For all species, cases in which age or sex were unknown were dropped prior to ANOVA.

	Sex			Age			Cohort			
	<i>n</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>F</i>	<i>P</i>	<i>n</i>	<i>df</i>	<i>F</i>	<i>P</i>
Northern Harrier	30	3.3	0.08	30	<b>7.5</b>	<b>0.01</b>	30	3	<b>2.9</b>	<b>0.05</b>
Sharp-shinned Hawk	392	0.2	0.69	392	<b>542.9</b>	<b>0.00</b>	392	3	<b>181.3</b>	<b>0.00</b>
Northern Goshawk	222	0.1	0.75	222	<b>8.3</b>	<b>0.00</b>	222	3	<b>3.1</b>	<b>0.03</b>
Long-eared Owl	14	0.0	0.95	83	1.5	0.22	12	2	0.1	0.92
American Kestrel	71	0.6	0.44	58	<b>5.3</b>	<b>0.02</b>	58	3	2.3	0.09
Merlin	137	<b>6.8</b>	<b>0.01</b>	137	<b>86.0</b>	<b>0.00</b>	137	3	<b>31.5</b>	<b>0.00</b>



**FIGURE 1.** Geometric mean feather mercury (Hg) concentration ( $\mu\text{g g}^{-1}$  fw) by sex for (A) hatch year birds (juvenile) and (B) after hatch year (adult) raptors banded at Hawk Ridge, Duluth, Minnesota, USA, 2009–2012. Data are shown for species with a total sample size (all ages)  $>20$ . Sample size is given above each bar and error bars represent geometric standard deviation.

The best linear model for ln(feather Hg) concentration for Sharp-shinned Hawk, receiving 65% of  $\text{AIC}_c$  weight ( $w_i$ ), included age and year as additive effects (Table 4). The next-best models, together receiving about 25% of  $w_i$ , included a sex\*age interaction (Table 4). Coefficients and 95% confidence limits (CL) for these 6 models indicate that

age received the highest  $\text{AIC}_c$  weight among all variables, followed closely by year (Table 5). Although the sex\*age interaction was selected in 2 of the top 3 models, neither the interaction, nor the main effect of sex, was bounded away from zero by the confidence limits. The year effect in the top 2 models, which included Year only as an additive effect, was bounded away from zero and indicated a decline in feather Hg over the 4 years of sampling. Ln(feather Hg) concentrations were higher in Sharp-shinned Hawk caught in 2009 compared to the 2010–2012 sampling years, and this was true for both age classes (Table 6).

For Merlin, 4 models were required to account for 99% of model weight, of which the top 3 received almost equal weight (Table 7). Date and age were included in all 4 of the top models. In general, coefficients suggested increasing ln(feather Hg) with age, although presence of an interaction term, such as date, could reverse this relationship (Table 8). Male Merlin tended to have lower ln(feather Hg) concentrations than females and this effect was generally bounded away from zero by the 95% confidence limit. However, year showed little effect on ln(feather Hg) in Merlin, with the 95% confidence limit typically encompassing zero. In general, the top models suggested slightly increasing ln(feather Hg) concentration in Merlin with date as the season progressed (Figure 2). Variable importance values calculated using  $\text{AIC}_c$  reinforced these patterns with age and date receiving considerable support, followed by sex, and lastly year (Table 8).

There was considerable overlap of  $\delta^{13}\text{C}$  values determined for Merlin and Sharp-shinned Hawk (Figure 3). Mean  $\delta^{13}\text{C}$  values for the 2 species were  $-21.8\text{‰}$  (range:  $-24.1\text{‰}$  to  $-16.2\text{‰}$ ) and  $-22.0\text{‰}$  (range:  $-23.6\text{‰}$  to  $-20.7\text{‰}$ ), respectively. These  $\delta^{13}\text{C}$  values were consistent across age and sex cohorts and suggest that individuals sampled were feeding from a terrestrial-based food web. Measured  $\delta^{13}\text{C}$  values were not significantly correlated with ln(feather Hg) for either Merlin or Sharp-shinned Hawk.

The mean  $\delta^{15}\text{N}$  value for Merlin (all age classes pooled) was  $7.0\text{‰}$  (range:  $3.6\text{‰}$  to  $13.8\text{‰}$ ) while that for Sharp-shinned Hawk (all age classes pooled) was  $6.2\text{‰}$  (range:  $4.0\text{‰}$  to  $9.0\text{‰}$ ) (Figure 3). The  $\delta^{15}\text{N}$  values for Merlin were positively correlated with ln(feather Hg) ( $r = 0.29$ ,  $P < 0.05$ ) across all pooled age and sex classes. For Sharp-shinned Hawk, feather  $\delta^{15}\text{N}$  values were

**TABLE 4.** Sample-size adjusted Akaike information criterion scores ( $\Delta\text{AIC}_c$ ),  $\text{AIC}_c$  weights ( $w_i$ ), and adjusted  $R^2$  values for Sharp-shinned Hawk banded at Hawk Ridge, Duluth, Minnesota, USA, 2009–2012. All models relate  $\ln(\text{feather Hg})$  to the factors and covariates listed. Date is the ordinal day from 1 to 365; year is a factor that takes values 2009, 2010, 2011, and 2012; sex is either male or female; age is either hatch year (HY) or after-hatch year (AHY).  $K$  is the number of estimated parameters in each model,  $w_i$  is the relative likelihood of the model, as measured by Akaike weights. Models with interaction terms always include the associated main effects.  $N = 392$ . Minimum  $\text{AIC}_c = 520.8$ . Models presented are the minimal model set accounting for at least 99% of  $\text{AIC}_c w_i$ .

Model	$K$	$\Delta\text{AIC}_c$	$w_i$	CumWt	Adjusted $R^2$
Age + Year	4	0.0	0.65	0.65	0.59
Year + Sex*Age	6	3.2	0.13	0.79	0.59
Date*Year + Sex*Age	8	3.3	0.12	0.91	0.59
Date*Age	5	5.5	0.04	0.95	0.59
Age + Date	4	6.2	0.03	0.98	0.59
Date + Sex*Age	6	8.8	0.01	0.99	0.59

also positively correlated with  $\ln(\text{feather Hg})$  ( $r = 0.30$ ,  $P < 0.01$ ). These correlations were driven primarily by significant correlations between  $\ln(\text{feather Hg})$  and  $\delta^{15}\text{N}$  in HY birds of both species. The relationship was not significant for AHY birds alone for either species, though the sample size was small for AHY Merlin ( $n = 19$ ).

We analyzed 40 duplicate breast feathers. Mean (SD) percent relative difference (PRD) across all duplicates was 16.0% (20.3%) and the Pearson product-moment correlation statistic ( $r$ ) was 0.98 ( $P < 0.05$ ). For HY birds ( $n = 26$ ), the mean PRD was 7.2% (10.2%),  $r = 0.99$  ( $P < 0.001$ ). For AHY birds ( $n = 13$ ), the mean PRD was 33.4% (25.0%) and  $r = 0.98$  ( $P < 0.001$ ). One individual with duplicate feathers sampled was of unknown age.

## DISCUSSION

Although mercury contamination in birds has been an issue of concern for decades (Scheuhammer et al. 2007, 2011; Whitney and Cristol 2017b, Evers 2018), Hg exposure patterns in fall migrant North American raptor species remain poorly understood (Bourbour et al. 2019). Feathers of HY birds sampled during fall migration are grown on the breeding grounds during the previous summer months. Mercury concentrations in HY raptor feathers represent an index of Hg exposure during the period over which they were grown during nestling development (Ackerman et al. 2011). The interpretation of Hg concentrations in AHY birds is more complicated. Although adult raptors molt a substantial fraction of their breast feathers on the breeding grounds (e.g., Bildstein and Meyer 2000, Warkentin et al. 2005), body burdens of MeHg reflect a bird's entire exposure history. Mercury accumulated in one year can carry over to the next and contribute to future feather Hg concentrations (Ofukany et al. 2012, LaVoie et al. 2014). This may be especially important for older individuals at higher risk to MeHg (Evers et al. 1998b) because molt does not reduce the whole-body MeHg burden to zero (Burger 1993). Exposure also varies seasonally for many migratory species (Edmonds et al. 2010, Ofukany et al. 2012). Recent

studies have shown that inter-feather variability in Hg concentration may increase uncertainty in the interpretation of study results when only a single feather is analyzed (Cristol et al. 2012, Peterson et al. 2019) as we have done. However, a comparison of duplicate feathers in a subset of birds showed less variation among feathers in our samples than has been reported elsewhere (mean relative difference  $< 50\%$ ), particularly for HY birds. Our analyses also showed strong correlations among duplicates, suggesting that feather Hg concentrations are a consistent index of Hg exposure. Nevertheless, our results also support recent recommendations to control and/or account for inter-feather variability when using feathers for Hg analysis and monitoring (Cristol et al. 2012, Peterson et al. 2019). Recommendations for the number of feathers to sample in relation to bird size, feather size, and study goals are provided by Peterson et al. (2019).

## Age Patterns

Measured Hg concentrations in feathers from AHY birds were higher than those in HY birds for all species except Long-eared Owl and Cooper's Hawk. This finding is consistent with previous reports of a positive relationship between tissue Hg and age for a variety of birds, including raptors (Evers et al. 1998a, Ackerman et al. 2011, Kwaansa-Ansah et al. 2015, Bourbour et al. 2019). However, other studies have reported a negative (Tartu et al. 2014) or no relationship (Majidi et al. 2015) between tissue Hg and age class. Thus, our failure to detect a positive relationship in Long-eared Owl and Cooper's Hawk is not unusual. One challenge with collecting breast feathers from AHY birds is to be confident that the feathers collected are not remaining juvenile feathers. It is easier to identify and collect AHY feathers from birds that have distinguishably different HY and AHY breast feathers, such as accipiters, than from species that have similar or indistinguishable HY and AHY breast feathers. While this is a possible confounding factor, the significant difference in Hg concentrations between HY and AHY individuals for most species suggests that most feathers sampled from AHY birds were AHY feathers.

**TABLE 5.** Coefficients and 95% confidence limits for Sharp-shinned Hawk linear models of  $\ln(\text{feather Hg})$  in relation to factors and covariates. Only the 6 best models, accounting for over-99% of model weight, are presented. Columns are models and rows are coefficients. Blank cell indicates coefficient did not occur in model.  $w_i$  are AIC<sub>c</sub> weights.

	Age + Year	Year + Sex*Age	Date*Year + Sex*Age	Date*Age	Age + Date	Date + Sex*Age	Var. Wt
Intercept	160 (70, 249)	157 (66, 247)	507 (-1120, 2135)	-1.46 (-2.68, -0.2)	-0.78 (-1.69, 0.13)	-0.9 (-1.89, 0.09)	1
Age*HY	1.15 (1.06, 1.25)	1.17 (1.05, 1.3)	1.14 (1.01, 1.27)	2.64 (0.77, 4.5)	1.07 (0.97, 1.17)	1.12 (0.99, 1.25)	1
SexM		0.06 (-0.07, 0.19)	0.03 (-0.1, 0.17)			0.05 (-0.09, 0.18)	0.27
Date			-1.35 (-7.35, 4.65)	0.01 (0, 0.01)	0 (0, 0.01)	0 (0, 0.01)	0.20
Year	-0.08 (-0.12, -0.03)	-0.08 (-0.12, -0.03)	-0.25 (-1.06, 0.56)				0.91
Date*Year			0 (0, 0)				0.12
SexM*Age*HY		-0.04 (-0.23, 0.15)	-0.07 (-0.26, 0.12)			-0.12 (-0.31, 0.07)	0.27
Model Weight	0.65	0.13	0.12	0.04	0.03	0.01	

**Sex Patterns**

Mercury concentrations in feathers were significantly higher in female Merlin than in males. For the other species examined, there were no significant differences in Hg concentration related to sex, which is consistent with previous findings in raptors (Barnes et al. 2019, Bourbour et al. 2019). Reverse sexual dimorphism is greater in accipiters, such as Sharp-shinned Hawk, than other raptor taxa (Snyder and Wiley 1976). For the Sharp-shinned Hawk, sex was a poor predictor of Hg concentration ( $P = 0.91$ ). Therefore, like age, the relationship between sexual size dimorphism and MeHg accumulation may not be simple. Complicating these patterns is the fact that females eliminate MeHg through egg laying, temporarily lowering blood MeHg concentrations (Becker 1992, Lewis et al. 1993, Heinz and Hoffman 2004, Ackerman et al. 2007). Bioenergetic considerations may also play a role because MeHg exposure is largely determined by the amount of contaminated food consumed. If, on average, the weight-normalized consumption rate (g food per day per g body weight) for each sex is approximately the same, differences in size would be offset by comparable differences in the mass of food consumed, resulting in similar whole-body MeHg concentrations (assuming similar rates of elimination and selection of similar prey items by males and females). Conversely, if the consumption rate for one of the sexes is higher (e.g., due to higher levels of activity or the energetic demands of reproduction), then MeHg concentrations achieved in these birds may be higher than those achieved in the other sex, regardless of sex-specific differences in size.

**Food Selection**

Differences in feather Hg concentrations among raptor species were expected to reflect differences in the extent to which they fed in aquatic vs. terrestrial food webs as well as their relative trophic position. More specifically, we examined whether an aquatic influence in the diet could explain elevated Hg concentrations in Sharp-shinned Hawk and Merlin. Assuming a typical  $\delta^{13}\text{C}$  trophic discrimination (+2.2‰) and an effective trophic level of between 3 and 4, the observed  $\delta^{13}\text{C}$  values suggested that both species were feeding primarily in terrestrial-based food webs (i.e. a diet source of approximately -28‰ to -30‰). If a freshwater food web influence had been important, there should have been lower (i.e. more negative)  $\delta^{13}\text{C}$  values associated with higher Hg concentrations. In comparison,  $\delta^{13}\text{C}$  values of freshwater-dependent species such as Common Loon (blood, -27‰; Burgess and Hobson 2006), Lesser Scaup (*Aythya affinis*; muscle, -26‰; Gurney et al. 2017), and Herring Gull (*Larus argentatus*; eggs, less than -24‰; Hebert et al. 2009), are far lower than values determined for Sharp-shinned Hawk (-22.0‰) and Merlin (-21.9‰) in this study. Beyond diet source, other factors that affect tissue-diet discrimination include metabolic activity and tissue type (Bearhop et al. 2002). The  $\delta^{13}\text{C}$  value calculated

**TABLE 6.** Sample size ( $n$ ), mean date, geometric mean feather Hg ( $\mu\text{g g}^{-1}$  fw) and confidence limits on the geomean by year for hatch year (HY) and after hatch year (AHY) Sharp-shinned Hawk sampled at Hawk Ridge, Duluth, Minnesota, USA.

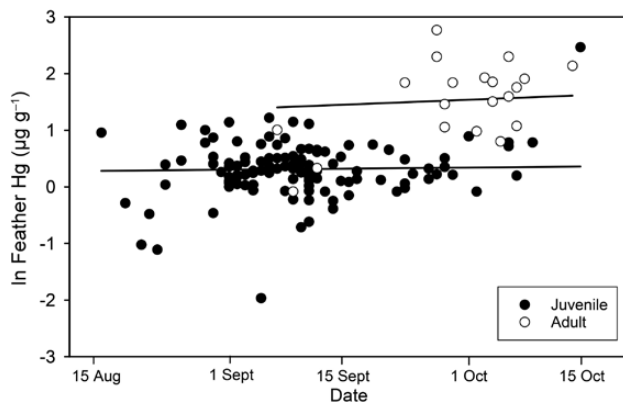
Year	Age	$n$	Date	GeoMean Hg	Lower 95% CL	Upper 95% CL
2009	HY	35	Oct 2	1.95	1.62	2.35
2010	HY	69	Sep 12	1.29	1.20	1.38
2011	HY	37	Sep 19	1.37	1.23	1.52
2012	HY	56	Sep 17	1.32	1.18	1.48
2009	AHY	26	Oct 4	5.92	4.97	7.05
2010	AHY	13	Sep 30	2.99	2.55	3.52
2011	AHY	81	Sep 24	4.19	3.73	4.70
2012	AHY	75	Oct 2	4.22	3.77	4.73

**TABLE 7.** Sample-size adjusted Akaike information criterion scores ( $\Delta\text{AIC}_c$ ),  $\text{AIC}_c$  weights ( $w_i$ ), and adjusted  $R^2$  values for Merlin banded at Hawk Ridge, Duluth, Minnesota, USA, 2009–2012. Models relate  $\ln(\text{feather Hg})$  to specified factors and covariates. Date is the ordinal day from 1 to 365; year is a factor that takes values 2009, 2010, 2011, and 2012; sex is either male or female; age is either hatch year (HY) or after hatch year (AHY).  $K$  is the number of estimated parameters in each model;  $w_i$  is the relative likelihood of the model, as measured by Akaike weights. Models with interaction terms always include the associated main effects.  $n = 137$ . Minimum  $\text{AIC}_c = 211.6$ . Models presented are the minimal model set accounting for at least 99% of  $\text{AIC}_c w_i$ .

Model	$K$	$\Delta\text{AIC}_c$	$w_i$	CumWt	Adjusted $R^2$
Date + Sex*Age	6	0.0	0.32	0.32	0.45
Date*Year + Sex*Age	8	0.1	0.31	0.63	0.46
Date*Age	5	0.1	0.30	0.93	0.44
Age + Date	4	3.5	0.06	0.99	0.42

**TABLE 8.** Coefficients and 95% confidence limits for Merlin linear models of  $\ln(\text{feather Hg})$ . Only the 4 best models, accounting for over 99% of model weight, are presented. Columns are models and rows are coefficients. Blank cell indicates coefficient did not occur in model. Wts are  $\text{AIC}_c$  weights.

	Date + Sex * Age	Date * Year + Sex * Age	Date * Age	Age + Date	Var. Wt.
Intercept	-3.09 (-5.1, -1.08)	1227 (-4239, 6692)	-2.14 (-4.29, 0.01)	-3.03 (-5.08, -0.98)	1.00
AgeAHY	0.89 (0.53, 1.26)	0.86 (0.5, 1.23)	-6.99 (-13.65, -0.33)	0.96 (0.66, 1.25)	1.00
SexM	-0.25 (-0.44, -0.06)	-0.26 (-0.44, -0.07)			0.64
Date	0.01 (0.01, 0.02)	-3.8 (-25.28, 17.68)	0.01 (0, 0.02)	0.01 (0.01, 0.02)	0.99
Year		-0.61 (-3.33, 2.11)			0.31
Date:Year		0 (-0.01, 0.01)			0.31
SexM:AgeAHY	0.04 (-0.45, 0.52)	0.04 (-0.44, 0.52)			0.63
Model Wt.	0.320	0.306	0.304	0.056	

**FIGURE 2.** Relationship of  $\ln(\text{Hg})$  ( $\mu\text{g g}^{-1}$  fw) to date by age class in Merlin. All feathers were collected at Hawk Ridge, Duluth, Minnesota, USA, 2009–2012. Feather Hg concentrations did not vary significantly with date in either hatch year or adult birds.

in the present study for Sharp-shinned Hawk is somewhat higher than the mean value ( $-25.6\text{‰}$ ) given previously by [Hobson \(1999\)](#). The  $\delta^{15}\text{N}$  value ( $7.1\text{‰}$ ) reported by [Hobson \(1999\)](#) for this species is similar to that determined in this study.

For both Sharp-shinned Hawk and Merlin, AHY birds had higher  $\delta^{15}\text{N}$  values than HY birds. Moreover, in multiple Sharp-shinned Hawk sex and age classes, higher  $\delta^{15}\text{N}$  values were significantly correlated with higher Hg concentrations, supporting the idea that trophic position is an important driver of MeHg exposure. Both species are known to prey extensively on birds. [Bourbour et al. \(2019\)](#) also found that raptors consuming predominantly avian prey had higher concentrations of total Hg than raptors not consuming a high percentage of avian prey. Songbirds that consume a high proportion of spiders or other predatory invertebrates may have elevated tissue concentrations



of total Hg (Jackson et al. 2015). Additional studies have shown that spiders may contain high concentrations of either total or methyl Hg (Cristol et al. 2008, Gann et al. 2015). Therefore, consumption of insectivorous songbirds may help explain higher Hg concentrations in some individual Sharp-shinned Hawk and Merlin (Jackson et al. 2011, 2015).

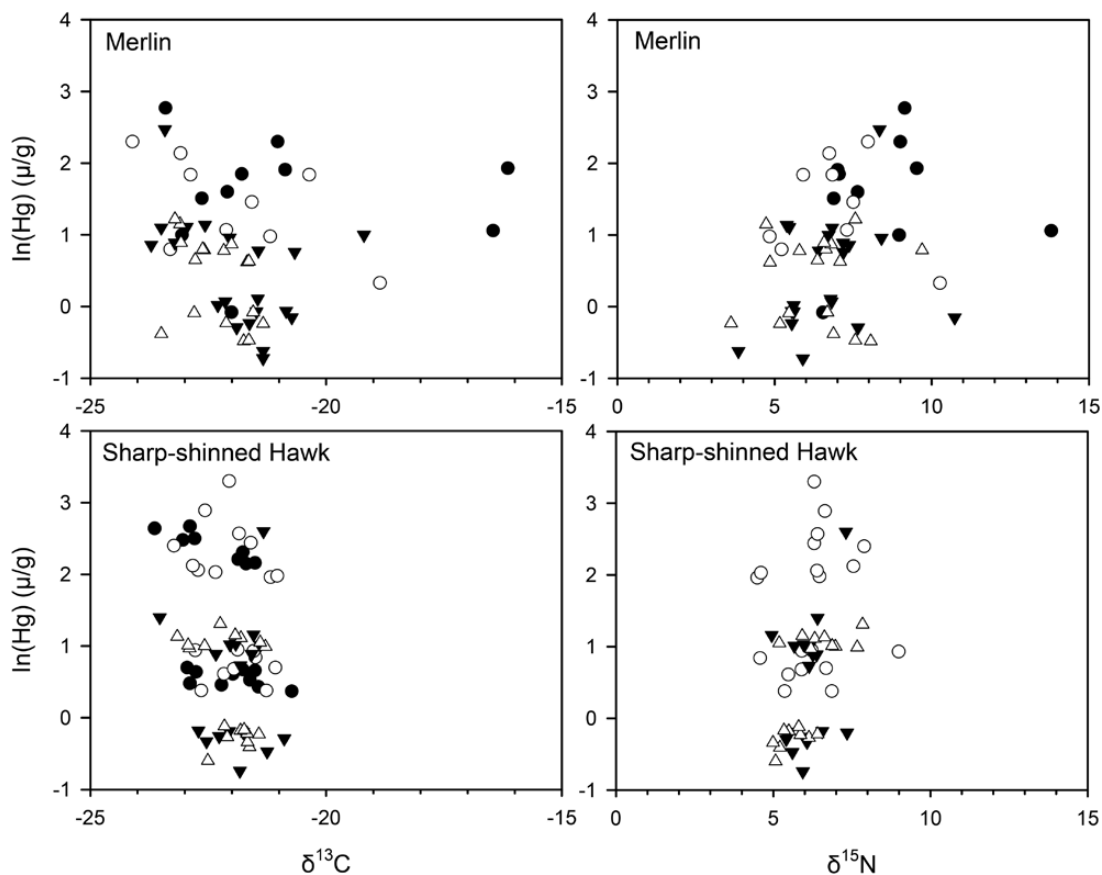
### Temporal Differences

In our analyses, date was one of the most important covariates in models of feather Hg concentration in Merlin. Bourbour et al. (2019) found a significant relationship between feather Hg and ordinal day in Red-tailed Hawks. Previous studies have noted that HY Merlin migrate earlier than AHY birds (Mueller et al. 2000). Therefore, date was likely another index to age. This relationship is also supported by our capture rates, with most HY birds caught before October 1 and most AHY birds caught after September 20 (Figure 2). However, this relationship may be complicated by many factors including distance between breeding grounds and Duluth, Minnesota, latitude of breeding grounds, and potential relationships between

size and age of individuals and migratory departure date. Date was not an important predictor variable in the Sharp-shinned Hawk models. Instead, year was more important than ordinal day. Sharp-shinned Hawk captured in 2009 had the highest Hg concentrations, whereas those captured in 2010 had the lowest Hg concentrations. These patterns are consistent for both age classes. The basis for these observed year-to-year differences in Hg concentrations is unclear. It is possible, however, that breeding populations sampled in different years originated from different regions of the United States and Canada, and experienced different MeHg exposure. Alternatively, annual differences in Hg concentrations could be related to variation in prey base among years.

### Feather Mercury Comparisons in Raptors

On average, feather Hg concentrations determined in this study are similar to feather residues reported for other terrestrial raptors and lower than feather Hg concentrations reported for piscivorous raptors (Table 9). Peregrine Falcons consume prey associated with both aquatic and terrestrial environments (White et al. 2002) and this may



**FIGURE 3.** Plot of  $\delta^{13}\text{C}$  (left) and  $\delta^{15}\text{N}$  (right) values against  $\ln(\text{Hg})$  concentrations ( $\mu\text{g g}^{-1}$  fw) for Merlin (top) and Sharp-shinned Hawk (bottom) sampled at Hawk Ridge, Duluth, Minnesota, USA. Data are identified by age and sex cohort. Open triangle = hatch year male, closed triangle = hatch year female, open circle = after hatch year male, and closed circle = after hatch year female.

**TABLE 9.** Reported feather Hg concentrations for raptors from previous studies. Osprey = *Pandion haliaetus*, Sparrowhawk = *Accipiter nisus*, Bald Eagle = *Haliaeetus leucocephalus*, Red-tailed Hawk = *Buteo jamaicensis*, Prairie Falcon = *Falco mexicanus*.

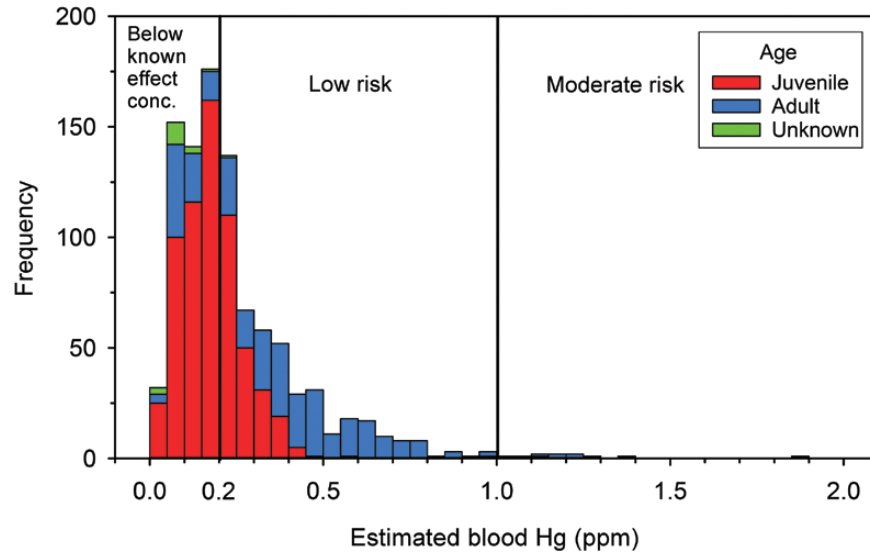
Species	Age	Average	Range	Article	Article Location	Feather Type
Osprey	Nesting	6.92 ug g <sup>-1</sup>	0.33–45.79 ug g <sup>-1</sup>	Rumbold et al. 2017	South Florida	body
Osprey	Adult	17.8 ug g <sup>-1</sup> dw	0.38–93.65 ug g <sup>-1</sup>	Rumbold et al. 2017	South Florida	body or fallen primary
Northern Harrier	Juvenile	0.75 ug g <sup>-1</sup>	95% CI 0.56–0.94 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Sparrowhawk	Unspecified	1.03 ug g <sup>-1</sup> dw	± 0.08 SD/SE	Zolfaghari et al. 2007	Iran	secondaries
Sparrowhawk	Unspecified	1.70 ug g <sup>-1</sup> dw	± 0.0006 SD/SE	Zolfaghari et al. 2007	Iran	rectrices
Sharp-shinned Hawk	Adult	4.35 ug g <sup>-1</sup>	95% CI 3.47–5.23 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Cooper's Hawk	Unspecified	2.35 ug g <sup>-1</sup>	95% CI 1.37–3.32 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Northern Goshawk	Unspecified	1.00 ug g <sup>-1</sup> dw	± 0.15 SD/SE	Zolfaghari et al. 2007	Iran	secondaries
Northern Goshawk	Unspecified	1.70 ug g <sup>-1</sup> dw	± 1.26 SD/SE	Zolfaghari et al. 2007	Iran	rectrices
Bald Eagle	Adult	21 ug g <sup>-1</sup> dw	0.20–66 ug g <sup>-1</sup>	Bowerman et al. 1994	Great Lakes	body
Bald Eagle	Adult	15.84 ug g <sup>-1</sup> dw	0.54–75.05 ug g <sup>-1</sup>	Bowerman et al. 1994	Great Lakes	breast
Red-shouldered Hawk	Unspecified	1.94 ug g <sup>-1</sup>	95% CI 0.75–3.14 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Red-tailed Hawk	Adult	0.56 ug g <sup>-1</sup>	95% CI 0.44–0.67 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
American Kestrel	Unspecified	0.57 ug g <sup>-1</sup>	95% CI 0.30–0.83 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Merlin	Juvenile	1.75 ug g <sup>-1</sup>	95% CI 1.20–2.30 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Peregrine Falcon	Nesting	3.76 ug g <sup>-1</sup> fw	0.12–49.64 ug g <sup>-1</sup>	Barnes and Gerstenberger 2015	Southern Nevada	primaries and/or axillaries
Peregrine Falcon	Adult	12.19 ug g <sup>-1</sup>	0.93–42.54 ug g <sup>-1</sup>	Barnes and Gerstenberger 2015	Southern Nevada	primaries and/or axillaries
Peregrine Falcon	Unspecified	3.93 ug g <sup>-1</sup>	95% CI 1.76–6.11 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast
Prairie Falcon	Unspecified	0.41 ug g <sup>-1</sup>	95% CI 0.16–0.67 ug g <sup>-1</sup>	Bourbour et al. 2019	California	breast

help explain their relatively high feather concentrations reported in previous studies. However, the limited number of Peregrine Falcons sampled in this study showed low feather Hg concentrations, comparable to other terrestrial raptors, although only HY birds were sampled. Accipiters, such as Northern Goshawk (*Accipiter gentilis*) and Sharp-shinned Hawk, tend to prey on terrestrial-associated birds (Squires and Reynolds 1997, Bildstein and Meyer 2000). Our findings for accipiters and buteos were similar to previous results (Table 9).

### Comparisons of Adverse Effect Concentrations

The potential for adverse effects of MeHg on avian wildlife has been evaluated in 3 recent reviews (Ackerman et al. 2016, Whitney and Cristol 2017b, Evers 2018). Numerous authors have correlated measured total Hg concentrations in tissues of field-collected animals with observed effects. This information has been used, in turn, to establish residue-based effect thresholds. Additional controlled laboratory exposures have been conducted to estimate effect thresholds expressed as an administered dose (e.g., in egg injection studies) or measured MeHg concentration in the diet. Collectively, the reviewed studies document a wide range of negative impacts including reduced reproductive success, behavioral changes, neurological effects, and various physiological and immune responses. Based on these and similar efforts, MeHg is now known to adversely affect the reproductive success of many bird populations across multiple foraging guilds, habitat types, and geographic areas.

A complicating factor in determining the risk of MeHg to birds is that species may vary in their sensitivity to an accumulated residue or delivered dose, potentially based on foraging guilds and phylogeny. For example, egg injection studies indicate that adverse effects on embryo survival and hatching success in songbirds and some raptors (e.g., Osprey and American Kestrel) occur at lower dose levels than those that cause similar effects in waterbirds (Orders Gaviiformes, Anseriformes, and Pelecaniformes; Heinz et al. 2009). The interpretation of such studies is complicated, however, by the unnatural nature of this dosing route. Perhaps the best studied of all birds with respect to adverse effects of MeHg is the Common Loon. Effect thresholds, expressed as MeHg concentrations in fish, have been established based on in situ observations (Evers et al. 2003, 2008; Burgess and Meyer 2008, Depew et al. 2012) and laboratory feeding studies (Kenow et al. 2003, 2010, 2011). The lowest proposed effect threshold (0.1  $\mu\text{g g}^{-1}$  fw) for dietary Hg was developed in adult Common Loons (Depew et al. 2012). Somewhat higher thresholds were given for significant reproductive impairment (0.18  $\mu\text{g g}^{-1}$  fw) and reproductive failure in wild adult loons (0.4  $\mu\text{g g}^{-1}$  fw). By comparison to the Common Loon, MeHg effects data for raptors are much more limited. Female American



**FIGURE 4.** Histogram of estimated total blood Hg concentrations by risk category for all individuals ( $n = 966$ ) sampled at Hawk Ridge, Duluth, Minnesota, USA (2009–2012). Risk categories are from Ackerman et al. (2016). Blood Hg concentrations were predicted using an empirical model that relates Hg concentrations in feathers to those in blood (Eagles-Smith et al. 2008).

Kestrels were exposed for approximately 16 weeks to MeHg in the diet and followed to evaluate effects on reproduction (Albers et al. 2007). The number of fledglings and the percent of nestlings fledged was reduced markedly at  $0.3 \mu\text{g g}^{-1}$  fw, while egg production, incubation performance, and the percent of eggs hatched declined between 1.2 and  $1.7 \mu\text{g g}^{-1}$  fw. Based on this information, it appears that effect thresholds for MeHg in the American Kestrel and Common Loon do not differ substantially. Presently, however, effect thresholds for other raptors are largely unknown.

In an attempt to estimate MeHg risk to raptors migrating through the Central flyway, we used an empirically based model given by Eagles-Smith et al. (2008) to estimate Hg concentrations in blood from those measured in feathers:  $\ln(\text{blood Hg}) = 0.749 \times \ln(\text{breast feather Hg}) - 1.855$ . This model was developed using data for pre-breeding waterbirds. Its application to raptors assumes that the processes responsible for sequestration of MeHg in developing feathers are the same in all birds. Predicted blood Hg concentrations were then compared with adverse effect thresholds recently proposed by Ackerman et al. (2016). These comparisons suggested that most of the individuals sampled in our study were at negligible or low risk to MeHg (Figure 4). However, a small number of AHY Merlin and Sharp-shinned Hawk exhibited predicted blood Hg concentrations associated with a moderate level of risk.

## CONCLUSION

For most of the sampled birds the predicted risk was low or negligible. Total Hg concentrations in breast feathers from raptors sampled during their fall migration through central

North America varied substantially within and among species. In general, Hg concentrations in AHY birds were higher than those in HY birds and appeared to vary by diet strategy, with bird-specialist species having higher concentrations than mammal-specialist species. Because Hg was correlated with ordinal day for Merlin, sampling over the entire period of migration may be required to accurately assess MeHg exposure in this and other raptors. An evaluation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in Merlin and Sharp-shinned Hawk suggested that both species feed primarily from a terrestrially associated food web and that the trophic level of individual birds is a major determinant of MeHg exposure. Measured concentrations of Hg in some AHY Merlin and Sharp-shinned Hawk suggest a moderate risk of adverse effects from MeHg exposure. It is currently unknown whether Hg residues in North American raptors are increasing, decreasing, or stable, and ongoing monitoring is essential to detect changes in these levels over time.

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**Ethics statement:** All birds were trapped and banded using standard protocols, as approved under the USFWS HRBO station permit. The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

**Author contributions:** E.R.K., M.A.E., G.J.N., D.C.E., C.R.D., J.W.N., and J.C.H. conceived the idea, design, experiment (supervised research, formulated question or hypothesis). E.R.K., F.N., D.C.E., C.R.D., J.W.N., and J.C.H. performed the experiments (collected data, conducted the research). E.R.K., M.A.E., G.J.N., C.R.D., J.W.N., and J.C.H. wrote the paper. M.A.E., D.C.E., C.R.D., and J.C.H. developed or designed methods. E.R.K., M.A.E., J.C.H., and Y.L. analyzed the data.

**Data depository:** Analyses reported in this article can be reproduced using the data provided by Keyel et al. (2020).

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**APPENDIX TABLE 10.** Geometric mean (Geomean), standard deviation (GeoSD), and minimum/maximum Hg feather concentrations by species, age, sex, and cohort for raptors sampled on Hawk Ridge, Duluth, Minnesota, USA.

Species	Age	<i>n</i>	Geomean	GeoSD	Min	Max
American Kestrel	AHY	5	1.02	2.44	0.38	2.19
American Kestrel	HY	53	0.44	2.18	0.03	3.32
American Kestrel	U	13	0.39	1.99	0.15	1.41
Broad-winged Hawk	HY	5	0.49	1.35	0.36	0.80
Cooper's Hawk	AHY	7	1.24	1.64	0.67	2.65
Cooper's Hawk	HY	4	0.50	2.36	0.17	1.02
Long-eared Owl	AHY	68	0.56	2.01	0.17	4.71
Long-eared Owl	HY	15	0.43	2.26	0.09	1.57
Long-eared Owl	U	5	0.30	1.16	0.24	0.35
Merlin	AHY	20	4.56	2.02	0.92	15.95
Merlin	HY	117	1.36	1.66	0.14	11.77
Northern Goshawk	AHY	26	1.34	2.73	0.30	16.41
Northern Goshawk	HY	196	0.83	2.16	0.15	4.46
Northern Harrier	AHY	5	2.36	2.36	0.74	6.18
Northern Harrier	HY	25	0.90	2.00	0.32	3.94
Peregrine Falcon	HY	8	1.18	2.01	0.40	2.76
Red-shouldered Hawk	HY	1	1.09	–	1.09	1.09
Sharp-shinned Hawk	AHY	195	4.30	1.68	1.45	27.18
Sharp-shinned Hawk	HY	197	1.41	1.53	0.40	13.52
Swainson's Hawk	HY	1	0.11	–	0.11	0.11
Species	Sex	<i>n</i>	GeoMean	GeoSD	Min	Max
American Kestrel	F	34	0.49	2.12	0.09	3.32
American Kestrel	M	37	0.42	2.31	0.03	1.84
Broad-winged Hawk	U	5	0.49	1.35	0.36	0.80
Cooper's Hawk	F	4	0.66	2.70	0.17	1.77
Cooper's Hawk	M	7	1.06	1.86	0.37	2.65
Long-eared Owl	F	7	0.41	2.08	0.24	1.80
Long-eared Owl	M	7	0.42	1.64	0.17	0.84
Long-eared Owl	U	74	0.54	2.08	0.09	4.71
Merlin	F	60	1.93	2.04	0.49	15.95
Merlin	M	77	1.43	1.89	0.14	9.98
Northern Goshawk	F	70	0.85	2.52	0.15	16.41
Northern Goshawk	M	152	0.89	2.14	0.16	6.37
Northern Harrier	F	13	1.41	2.30	0.46	6.18
Northern Harrier	M	17	0.84	2.04	0.32	3.67
Peregrine Falcon	F	3	1.81	1.71	0.99	2.76
Peregrine Falcon	M	5	0.91	2.01	0.40	2.45
Red-shouldered Hawk	U	1	1.09	–	1.09	1.09
Sharp-shinned Hawk	F	213	2.42	2.14	0.40	15.69
Sharp-shinned Hawk	M	179	2.50	2.01	0.55	27.18
Swainson's Hawk	U	1	0.11	–	0.11	0.11
Species	Cohort	<i>n</i>	Geomean	GeoSD	Min	Max
American Kestrel	AHYF	4	0.88	2.60	0.38	2.19
American Kestrel	AHYM	1	1.84	–	1.84	1.84
American Kestrel	HYF	17	0.51	2.03	0.09	3.32
American Kestrel	HYM	36	0.40	2.24	0.03	1.76
American Kestrel	UF	13	0.39	2.02	0.15	1.41
Broad-winged Hawk	HYU	5	0.49	1.34	0.36	0.80
Cooper's Hawk	AHYF	2	1.09	1.99	0.67	1.77
Cooper's Hawk	AHYM	5	1.31	1.63	0.67	2.65
Cooper's Hawk	HYF	2	0.41	3.43	0.17	0.97
Cooper's Hawk	HYM	2	0.61	2.05	0.37	1.02
Long-eared Owl	AHYF	5	0.46	2.37	0.24	1.80
Long-eared Owl	AHYM	6	0.40	1.70	0.17	0.84
Long-eared Owl	AHYU	57	0.58	2.01	0.19	4.71
Long-eared Owl	HYM	1	0.52	–	0.52	0.52
Long-eared Owl	HYU	14	0.42	2.32	0.09	1.57
Long-eared Owl	UF	2	0.31	1.2	0.27	0.35



**APPENDIX TABLE 10.** Continued

Species	Cohort	<i>n</i>	Geomean	GeoSD	Min	Max
Long-eared Owl	UU	3	0.29	1.18	0.24	0.33
Merlin	AHYF	11	4.95	2.12	0.92	15.95
Merlin	AHYM	9	4.13	1.94	1.39	9.98
Merlin	HYF	49	1.56	1.66	0.49	11.77
Merlin	HYM	68	1.24	1.62	0.14	3.38
Northern Goshawk	AHYF	14	0.86	3.51	0.30	16.41
Northern Goshawk	AHYM	12	1.31	1.93	0.48	6.37
Northern Goshawk	HYF	56	0.76	2.02	0.15	3.36
Northern Goshawk	HYM	140	0.86	2.14	0.16	4.46
Northern Harrier	AHYF	4	2.33	2.69	0.74	6.18
Northern Harrier	AHYM	1	2.46	–	2.46	2.46
Northern Harrier	HYF	9	1.13	2.02	0.46	3.94
Northern Harrier	HYM	16	0.79	1.97	0.32	3.67
Peregrine Falcon	HYF	3	1.81	1.71	0.99	2.76
Peregrine Falcon	HYM	5	0.91	2.01	0.40	2.45
Red-shouldered Hawk	HYU	1	1.09	–	1.09	1.09
Sharp-shinned Hawk	AHYF	107	4.31	1.72	1.45	15.69
Sharp-shinned Hawk	AHYM	88	4.30	1.62	1.46	27.18
Sharp-shinned Hawk	HYF	106	1.36	1.54	0.40	13.52
Sharp-shinned Hawk	HYM	91	1.48	1.52	0.55	5.99
Swainson's Hawk	HYU	1	0.11	–	0.11	0.11