



# Feather mercury concentrations in North American raptors sampled at migration monitoring stations

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## Abstract

We assessed total mercury (THg) concentrations in breast feathers of diurnal North American raptors collected at migration monitoring stations. For 9 species in the Pacific Flyway, we found species and age influenced feather THg concentrations whereas sex did not. Feather THg concentrations  $\mu\text{g/g}$  dry weight (dw) averaged (least squares mean  $\pm$  standard error) higher for raptors that generally consume  $> 75\%$  avian prey (sharp-shinned hawk *Accipiter striatus*:  $n = 113$ ;  $4.35 \pm 0.45 \mu\text{g/g}$  dw, peregrine falcon *Falco peregrinus*:  $n = 12$ ;  $3.93 \pm 1.11 \mu\text{g/g}$  dw, Cooper's hawk *Accipiter cooperii*:  $n = 20$ ;  $2.35 \pm 0.50 \mu\text{g/g}$  dw, and merlin *Falco columbarius*:  $n = 59$ ;  $1.75 \pm 0.28 \mu\text{g/g}$  dw) than for raptors that generally consume  $< 75\%$  avian prey (northern harrier *Circus hudsonius*:  $n = 112$ ;  $0.75 \pm 0.10 \mu\text{g/g}$  dw, red-tailed hawk *Buteo jamaicensis*:  $n = 109$ ;  $0.56 \pm 0.06 \mu\text{g/g}$  dw, American kestrel *Falco sparverius*:  $n = 16$ ;  $0.57 \pm 0.14 \mu\text{g/g}$  dw, prairie falcon *Falco mexicanus*:  $n = 10$ ;  $0.41 \pm 0.13 \mu\text{g/g}$  dw) except for red-shouldered hawks *Buteo lineatus*:  $n = 10$ ;  $1.94 \pm 0.61 \mu\text{g/g}$  dw. Feather THg concentrations spanning 13-years (2002–2014) in the Pacific Flyway differed among 3 species, where THg increased for juvenile northern harrier, decreased for adult red-tailed hawk, and showed no trend for adult sharp-shinned hawk. Mean feather THg concentrations in juvenile merlin were greater in the Mississippi Flyway ( $n = 56$ ;  $2.14 \pm 0.18 \mu\text{g/g}$  dw) than those in the Pacific Flyway ( $n = 49$ ;  $1.15 \pm 0.11 \mu\text{g/g}$  dw) and Intermountain Flyway ( $n = 23$ ;  $1.14 \pm 0.16 \mu\text{g/g}$  dw), and Atlantic Flyway ( $n = 38$ ;  $1.75 \pm 0.19 \mu\text{g/g}$  dw) averaged greater than the Pacific Flyway. Our results indicate that raptor migration monitoring stations provide a cost-effective sampling opportunity for biomonitoring environmental contaminants within and between distinct migration corridors and across time.

**Keywords** Raptor · Contaminants · Biomagnification · Biomonitoring · Migration · Methylmercury

## Introduction

Mercury (Hg) contamination in the environment is ubiquitous across the globe, present in remote ecosystems, and anthropogenic activities have contributed to its release (Fitzgerald et al. 1998; Obrist et al. 2018). Once in the environment, Hg can be transformed to methylmercury (MeHg), which is the form most biologically available and

biomagnified in food webs (Wiener et al. 2003), where even low levels of exposure in wildlife has been shown to affect immune system function, behavior, reproductive success, survival, cellular function, and is attributed to other abnormalities, especially during development (Wolfe et al. 1998; Ackerman et al. 2016a; Albers et al. 2007; Heinz et al. 2009). While the methylation processes of Hg are typically confined to aquatic ecosystems, MeHg can be transferred via trophic interactions into terrestrial ecosystems potentially leading to biomagnification in terrestrial food webs (Cristol et al. 2008; Rimmer et al. 2010; Becker et al. 2018).

Biomonitoring of sentinel wildlife species, such as apex predators, can assess biomagnified levels of MeHg in various food webs across space and time. Diurnal birds of prey (raptors; e.g., hawks/eagles (Accipitridae) and falcons (Falconidae), can be useful in this assessment because they occupy a variety of terrestrial habitats and feed at the top of

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various food webs around the world; their use as biosentinel for contamination is already common in Europe and Asia (Espín et al. 2016; Gómez-Ramírez et al. 2014; García-Seoane et al. 2017; Walker et al. 2008; Movalli 2000; Zolfaghari et al. 2007). In North America, MeHg exposure in terrestrial feeding raptors has received relatively little attention; previous studies highlight raptors that have a direct link to aquatic ecosystems, such as piscivores (e.g., bald eagles *Haliaeetus leucocephalus* and osprey *Pandion haliaetus*; Bowerman et al. 1994; DesGranges et al. 1998; Weech et al. 2006; Henny et al. 2010; Guigueno et al. 2012; DeSorbo et al. 2018), despite MeHg being prevalent in terrestrial food webs (Cristol et al. 2008).

Feather Hg concentrations represents dietary MeHg in the blood at the time of feather growth (Furness et al. 1986; Lodenius and Solonen 2013). Blood Hg concentrations during feather growth are influenced by both the recent dietary intake of MeHg and the redistribution of MeHg among tissues (Furness et al. 1986). While feathers provide a simple and less invasive sampling opportunity, it is important to emphasize that feather Hg concentration can vary widely within and among individual feathers and feather types (Cristol et al. 2012; Lodenius and Solonen 2013), warranting careful consideration when interpreting feather Hg concentrations. Sampling tissues such as blood or eggs can provide a more informative measure of local Hg exposure in birds, as Hg concentrations can more readily be translated into common toxicity benchmarks and represent more current Hg concentrations and risk (Ackerman et al. 2016a). However, collecting blood or egg samples is not always feasible, especially for rare or protected species (Ackerman et al. 2012), and sampling feathers from raptors for contaminant monitoring at multiple sites is more common and can more easily be adapted to collaborative research over large geographic regions (Smith et al. 2003).

When looking for large or coarse scale trends of Hg exposure, feathers offer an opportunity for broad interspecific comparisons, in addition to temporal and spatial contaminant studies (Keyel 2016; Bond et al. 2015; García-Seoane et al. 2017). Breast feathers are relatively easy to obtain from living birds and sampling of feathers is minimally invasive because collection does not impair flight (Furness et al. 1986; Dauwe et al. 2003; Espín et al. 2016, 2014). While use of blood samples for Hg exposure studies can be advantageous because of less intra-individual variation among repeated samples, sampling feathers at migration concentration points provides an opportunity to remotely monitor the Hg exposure raptors are experiencing on breeding grounds because juvenile feathers were grown during chick development at nest sites and adult feathers are similarly grown during the breeding season (see Bildstein and Meyer 2000, Smallwood and Bird 2002, Curtis et al. 2006, Preston and Beane 2009). The specific ecosystems

represented by these feather samples vary by species due to species-specific habitat preferences. For example, sharp-shinned hawk *Accipiter striatus* feathers will be broadly representative of the boreal and montane forests that this species occupies during the breeding season (Bildstein and Meyer 2000), while American kestrel *Falco sparverius* feathers reflect Hg exposure in their grassland and savannah breeding habitats (Smallwood and Bird 2002).

In this study, we used archived breast feathers to 1) quantify feather THg concentrations in diurnal raptor species in the Pacific Flyway and look at comparisons among 9 species, 2) assess temporal trends of Hg exposure spanning a 13-year period (2002–2014) in the Pacific Flyway for three raptor species, juvenile northern harrier *Circus hudsonius*, adult sharp-shinned hawk, and adult red-tailed hawk *Buteo jamaicensis*, and 3) investigate spatial patterns of Hg exposure among four distinct North American flyways (Pacific, Intermountain, Mississippi, and Atlantic) for a single raptor species, merlin *Falco columbarius*.

## Methods

### Sample collection and study design

We compiled archived breast feathers (plucked from approximately 2 cm below crop) collected from raptors banded during autumn migration, as collecting and archiving breast feathers is accepted as part of standard procedure during the banding process (Smith et al. 2003). Feathers were collected from individuals appearing to be healthy during the banding process. Based on the results of long-term band recovery data from North American raptor banding stations (Goodrich and Smith 2008, Hull et al. 2009, GGRO 2015), throughout this study we use migration monitoring stations located along known concentration points as representative of North American migration flyways (Pacific, Intermountain, Mississippi, and Atlantic).

To investigate differences in Hg exposure among raptor species in the Pacific Flyway, we analyzed archived feathers from 10 diurnal raptor species banded by the Golden Gate Raptor Observatory in the Marin Headlands of California. To reduce effect of interannual variation in Hg exposure, we primarily collected samples from within a single year (2006), however, we also included feathers from rarely sampled species or age classes from other years due to low sample sizes in any one specific year within the time frame of 2002–2014 (Tables 1 and 2). We classified age as either juvenile (hatch-year; <1 yr. old; have not undergone feather molt) or adult (after-hatch-year; >1 yr. old; have undergone feather molt). We categorized a species as a bird specialist if their diet typically consisted of over 75% avian prey (i.e., sharp-shinned hawk, Cooper's hawk *Accipiter cooperii*,

**Table 1** Model estimates for total mercury (THg) concentrations (µg/g dw) in raptor breast feathers from 10 species sampled in the Pacific Flyway

Family	Common name	n	Sample year(s)	(A) Full model (averaged over age and sex)			(B) Adults (averaged over sex)			(C) Juveniles (averaged over sex)			
				µg/g dw THg			µg/g dw THg			µg/g dw THg			
				LS Mean ± SE	95% CI	n	Sample year(s)	n	Sample year(s)	LS Mean ± SE	95% CI	n	Sample year(s)
<b>Accipitridae</b>													
	Northern Harrier	112	2002–2014	0.75 ± 0.10	0.56–0.94	9	2006, 2012–13	1.02 ± 0.17	0.68–1.35	103	2002–14	0.48 ± 0.04	0.39–0.56
	Sharp-shinned Hawk <sup>a</sup>	113	2002–2014	4.35 ± 0.45	3.47–5.23	102	2002–2014	5.92 ± 0.60	4.74–7.10	11	2006, 2009	2.77 ± 0.46	1.88–3.67
	Cooper's Hawk <sup>a</sup>	20	2006	2.35 ± 0.5	1.37–3.32	10	2006	3.20 ± 0.71	1.80–4.60	10	2006	1.50 ± 0.33	0.84–2.15
	Red-shouldered Hawk	10	2006	1.94 ± 0.61	0.75–3.14	–	–	–	–	10	2006	1.24 ± 0.37	0.52–1.96
	Broad-winged Hawk <sup>b</sup>	–	–	–	–	–	–	–	–	10	2006, 2012–14	0.31 <sup>b</sup>	0.24–0.41 <sup>b</sup>
	Red-tailed Hawk	109	2002–2014	0.56 ± 0.06	0.44–0.67	98	2006	0.76 ± 0.08	0.61–0.92	11	2006, 2007	0.36 ± 0.06	0.24–0.47
<b>Falconidae</b>													
	Merlin <sup>a</sup>	59	2006, 2007	1.75 ± 0.28	1.20–2.30	–	–	–	–	59	2006, 2007	1.12 ± 0.14	0.85–1.39
	American Kestrel	16	2006	0.57 ± 0.14	0.30–0.83	6	2006	0.77 ± 0.20	0.39–1.16	10	2006	0.36 ± 0.09	0.19–0.53
	Prairie Falcon	10	2002, 2006–07, 2010–13	0.41 ± 0.13	0.16–0.67	–	–	–	–	10	2002, 2006–07, 2010–13	0.26 ± 0.08	0.11–0.42
	Peregrine Falcon <sup>a</sup>	12	2003, 2006–10	3.93 ± 1.11	1.76–6.11	2	2003, 2008	5.36 ± 1.60	2.21–8.50	10	2006–07, 2009–10	2.51 ± 0.68	1.17–3.85

This data represents sample size (n), year(s) feathers were sampled, least squares mean ± standard error (LS Mean ± SE), and 95% confidence interval (CI). LS Mean ± SE and 95% CI were calculated using linear mixed-effects models with sample year as a random effect. (A) The full linear mixed-effects model includes species, age, and sex as factors, (B) the adult model includes species aged as after-hatch-year and sex, (C) the juvenile model includes species aged as hatch-year and sex

<sup>a</sup>Bird-specialists with diets that generally consist of >75% avian prey

<sup>b</sup>Broad-winged hawks were excluded from statistical models due to unknown sex. Broad-winged hawk data represents sample size, sample year, and geometric mean and 95% CI THg µg/g dw of breast feather THg back natural-log transformed using the delta method

**Table 2** Total mercury (THg) concentrations ( $\mu\text{g/g dw}$ ) in raptor breast feathers sampled in the Pacific Flyway

Family	Common name	(A) Adult female			(B) Adult male			(C) Juvenile female			(D) Juvenile male			
		n	Sample year(s)	$\mu\text{g/g dw THg}$ Mean $\pm$ SE	n	Sample year(s)	$\mu\text{g/g dw THg}$ Mean $\pm$ SE	n	Sample year(s)	$\mu\text{g/g dw THg}$ Mean $\pm$ SE	n	Sample year(s)	$\mu\text{g/g dw THg}$ Mean $\pm$ SE	
Accipitridae	Northern Harrier	4	2006, 2012-13	0.87 $\pm$ 0.42	5	2006, 2012	1.08 $\pm$ 0.47	59	2002-09, 2012-14	0.43 $\pm$ 0.04	44	2002, 2004-14	0.54 $\pm$ 0.06	
	Sharp-shinned Hawk <sup>a</sup>	96	2002-2014	6.14 $\pm$ 0.39	6	2006-07, 2009	5.05 $\pm$ 1.27	6	2006, 2009	1.96 $\pm$ 0.75	5	2006	0.78 $\pm$ 0.32	
	Cooper's Hawk <sup>a</sup>	5	2006	2.62 $\pm$ 1.24	5	2006	3.51 $\pm$ 1.65	5	2006	1.40 $\pm$ 0.36	5	2006	1.78 $\pm$ 0.45	
	Red-shouldered Hawk	-	-	-	-	-	-	-	5	2006	2.00 $\pm$ 1.03	5	2006	0.77 $\pm$ 0.39
	Red-tailed Hawk	93	2002-2014	0.67 $\pm$ 0.09	5	2006	1.39 $\pm$ 0.79	6	2006, 2007	0.28 $\pm$ 0.10	5	2006	0.94 $\pm$ 0.36	
Falconidae	Merlin <sup>a</sup>	-	-	-	-	-	-	22	2006, 2009	1.32 $\pm$ 0.22	37	2006, 2009	1.04 $\pm$ 0.13	
	American Kestrel	3	2006	0.43 $\pm$ 0.12	3	2006	0.44 $\pm$ 0.12	5	2006	0.48 $\pm$ 0.20	5	2006	0.54 $\pm$ 0.23	
	Prairie Falcon	-	-	-	-	-	-	5	2007, 2011-13	0.12 $\pm$ 0.06	5	2002, 2006, 2010-11	0.60 $\pm$ 0.32	
	Peregrine Falcon <sup>a</sup>	2	2003, 2008	3.86	-	-	-	5	2006, 2007	2.09 $\pm$ 1.03	5	2006-07, 2009-10	3.34 $\pm$ 1.65	
	-	-	-	-	-	-	-	-	-	-	-	-	-	

This data is organized by age (adult or juvenile) and sex (female or male) and represents sample size (n), year(s) feathers were sampled, and geometric mean  $\pm$  standard error (Mean  $\pm$  SE). Feather THg was calculated for (A) adult females of six species, (B) adult males of five species, (C) juvenile females of nine species, and (D) juvenile males of nine species. Broad-winged hawks are excluded from table due to unknown sex

<sup>a</sup> Bird-specialists with diets that typically consist of >75% avian prey

merlin, and peregrine falcon *Falco peregrinus*) or as a non-bird specialist if their diet typically consisted of less than 75% avian prey (i.e., northern harrier, red-shouldered hawk *Buteo lineatus*, broad-winged hawk *Buteo platypterus*, red-tailed hawk, American kestrel, and prairie falcon *Falco mexicanus*) based on published species accounts and field guides (Bildstein and Meyer 2000; Curtis et al. 2006; Warkentin et al. 2005; White et al. 2002; Smith et al. 2011; Dykstra et al. 2008; Goodrich et al. 2014; Preston and Beane 2009; Smallwood and Bird 2002; Steenhof 2013; Peeters and Peeters 2005). We determined sex of each sexually dimorphic raptor based on measurements of informative morphological characters, such as wing chord, tarsus depth, and hallux (Pitzer et al. 2008; GGRO 1998).

To investigate temporal trends in Hg exposure within the Pacific Flyway, we collected archived feathers of juvenile northern harrier, adult sharp-shinned hawk, and adult red-tailed hawk that spanned a 13-year period (2002–2014) and were sampled as migrants in the Marin Headlands, California. For adult red-tailed hawks only, we included additional samples in all years collected in the northern Central Valley of California ( $n = 58$ ; Yolo County, Solano County, Butte County, Colusa County) from November through February months, which based on population genetics and band recoveries are understood to be part of the same distinct population that use the Pacific Flyway as a migration corridor and are sampled in the Marin Headlands (Hull et al. 2009; Goodrich and Smith 2008).

To investigate geographic Hg exposure trends among four distinct raptor migration flyways in North America, we collected feathers from juvenile merlins in the Pacific, Intermountain (a defined migration corridor within the western Central Flyway; Goodrich and Smith 2008), Mississippi, and Atlantic flyways during fall migration of 2009. All Pacific Flyway samples were collected in the Marin Headlands, California, all Mississippi Flyway samples were collected in Duluth, Minnesota, and all Atlantic Flyway samples were collected in Cape May, New Jersey. Multiple banding stations contributed feather samples from the Intermountain Flyway (Chelan Ridge, Washington; Bonney Butte, Oregon; Boise, Idaho; Goshutes Mountains, Nevada; Commissary Ridge, Wyoming; Manzanos Mountains, New Mexico). Importantly, all feathers collected from juvenile merlins during migration represent breast feathers grown on the breeding grounds in the Boreal and Pacific Northwest region of North America (Warkentin et al. 2005). Feather samples from adult individuals were only available from the Intermountain and Mississippi flyways.

### Sample processing and mercury determination

We conducted total Hg (THg) analysis at the U.S. Geological Survey, Dixon Field Station Environmental Mercury

Laboratory. We washed all feathers individually in 1% Alconox<sup>®</sup> solution (Alconox, Inc.) and manually scrubbed each feather to remove any surface debris from the physical environment followed by rinsing with deionized water (Herring et al. 2017). We dried each feather at approximately 50 °C for 24–48 h and stored them in desiccators prior to THg analysis. We used THg concentration as an index of MeHg because Hg in feathers is almost entirely MeHg (Ackerman et al. 2016a). We determined THg concentrations on the Nippon MA-3000 Direct Mercury Analyzer (Nippon Instruments, College Station, Texas) following Environmental Protection Agency Method 7473 (U.S. EPA). We analyzed one feather per individual for all species to determine the THg concentration. The exception was American kestrels, for which we analyzed two feathers per bird due to the smaller feather size.

Quality assurance measures included analysis of a certified reference material (CRM; either dogfish muscle tissue [DORM] or lobster hepatopancreas [TORT] certified by the National Research Council of Canada, Ottawa, Canada), system blank, method blank, continuing calibration verification (CCV), duplicate, and a spiked duplicate with each batch.

Percent recoveries (mean  $\pm$  SD) for CRM and CCV was  $101.5 \pm 1.5\%$  ( $n = 73$ ) and  $102.0 \pm 1.6\%$  ( $n = 62$ ), respectively. The mean absolute relative percent difference for feather duplicates was  $15.0 \pm 26.3\%$  ( $n = 65$ ), and these did not represent true method duplicates but rather should be interpreted as differences between individual feathers because single feathers were analyzed for each duplicate. The mean absolute relative percent difference for matrix spike duplicates was  $0.7 \pm 0.3\%$  ( $n = 9$ ).

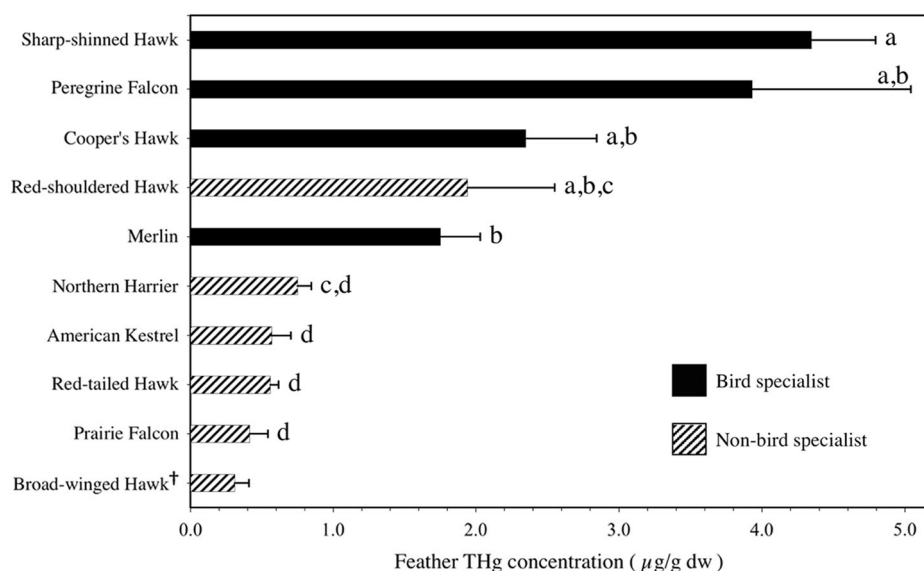
## Statistical analyses

We took the natural-log of feather THg concentrations ( $\mu\text{g/g}$  of dry wt.) to normalize data before analyses. We used R Studio V0.99.484 (R Studio Team 2015) for all statistical analyses. We conducted three separate analyses for THg concentrations determined in raptor feathers: 1) Multi-species comparisons within the Pacific Flyway, 2) Temporal trends over a 13-year period (2002–2014) for three species in the Pacific Flyway, and 3) Geographic trends for a single species (merlin) sampled in four North American flyways. We calculated all model estimates, Tukey pair-wise post-hoc tests, back-transformed data (delta method; Seber 1982), and fractional degrees of freedom (Satterthwaite method) using R Package 'lsmeans' (Lenth 2016). We calculated conditional  $R^2$  ( $R_c^2$ ) for describing the variance explained by factors for each model.

First, to test for differences among species, we used ANOVA for a linear mixed effects model that included species (9 species), age (juvenile or adult), and sex (male or female) as fixed effects and year as a random effect. Feather THg concentrations for broad-winged hawk were excluded from statistical analyses due to unknown sex, but we calculated geometric mean [95% CI]  $\mu\text{g/g}$  dry weight (dw) for reference. We used 95% CI Tukey pair-wise comparisons averaged over age and sex for post-hoc analyses. Interactions between species and age and between species and sex were not included in our ANOVA model due to rank deficiencies caused by uneven sample sizes in the archived feathers available.

Second, for temporal analyses (2002–2014) of juvenile northern harrier, adult sharp-shinned hawk, and adult red-tailed hawk we used ANOVA for linear models. For northern harrier and sharp-shinned hawk, we included year (continuous), passage date through migration monitoring site (continuous), and sex (male or female) as fixed effects. For red-tailed hawk, we included year (continuous), passage date through migration monitoring site (Marin Headlands) or sample collection date (Central Valley; continuous), sex (male or female), site (Marin Headlands or Central Valley), and an interaction between passage/sample collection date, and site as fixed effects. If site was a significant factor for feather THg concentrations in red-tailed hawks, we ran a separate analysis with only individuals sampled at the migration banding station in the Marin Headlands, CA to account for site, since sample collection date for red-tailed hawks in the Central Valley ranged from November to February and those sampled in the Marin Headlands ranged from August to early January. For raptors sampled in the Marin Headlands, passage date represents the date when feather samples were collected from an individual migrating through the migration monitoring site, and any effect from passage date may indicate differential Hg exposure among geographically or genetically distinct raptor populations (Goodrich and Smith 2008; Hull et al. 2009). For temporal figure and red-tailed hawk passage date figure, back-transformations, partial residuals, and confidence bands were calculated and displayed using R Package 'visreg' (Breheny and Burchett 2017).

Third, for the geographic analysis of juvenile merlin among the four North American flyways, we used ANOVA for linear mixed effects models using flyway and sex as fixed effects and site as random effect due to having multiple sampling sites within the Intermountain Flyway. As described above, migration monitoring sites have been previously documented to be representative of the broader migration flyway within which they are located (Goodrich and Smith 2008). We used 95% CI Tukey pair-wise



**Fig. 1** Least squares (LS) mean  $\pm$  standard error  $\mu\text{g/g dw}$  of breast feather THg concentrations in 9 North American raptor species sampled in the Pacific Flyway. THg concentrations are averaged over age and sex and estimated from a linear mixed-effects model with species, age, and sex as fixed effects, and year as a random effect. Species that do not share a lower-case letter are significantly different ( $p < 0.05$ )

comparisons averaged over sex for post-hoc analyses. Adults sampled in Intermountain and Mississippi flyways were analyzed using ANOVA separately.

## Results

### Multispecies results

We tested for differences of feather THg concentrations among 9 raptor species (Fig. 1; Tables 1 and 2) and found that species and age were significant predictors of THg, whereas sex was not (*Species*:  $F_{8, 450} = 45.48$ ,  $p < 0.001$ ; *Age*:  $F_{1, 450} = 25.83$ ,  $p < 0.001$ ; *Sex*:  $F_{1, 450} = 1.36$ ,  $p = 0.24$ ;  $R_c^2 = 0.53$ ). Tukey pair-wise comparisons (95% CI) revealed that sharp-shinned hawk averaged significantly higher ( $p < 0.001$ ) THg concentrations than all non-bird specialists except for red-shouldered hawk, and one bird specialist, merlin (Fig. 1). Peregrine falcons, Cooper's hawks, and merlins averaged significantly higher ( $p < 0.05$ ) THg concentrations than all non-bird specialists except for red-shouldered hawk (Fig. 1). Red-shouldered hawks averaged significantly higher ( $p < 0.05$ ) THg concentrations than red-tailed hawks, American kestrels, and prairie falcons (Fig. 1).

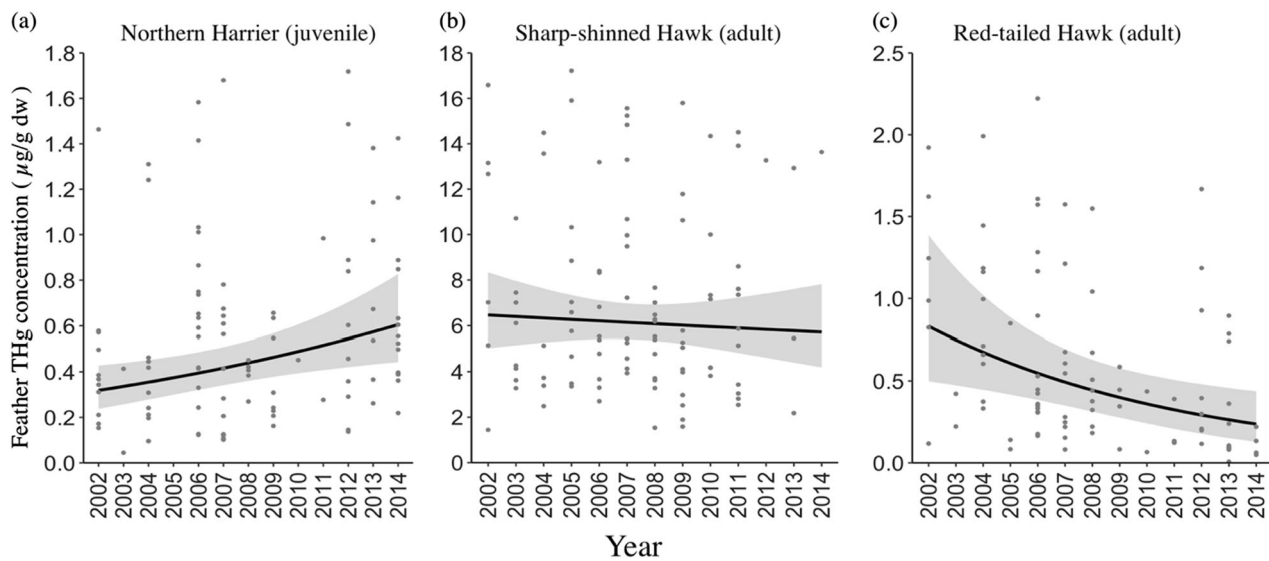
### Temporal results

We tested for temporal trends of Hg exposure in juvenile northern harrier, adult sharp-shinned hawk, and adult red-

tailed hawk sampled in the Pacific Flyway over a 13-year period (Fig. 2; Table 3). For juvenile northern harrier, we found that year was a significant predictor of THg, whereas passage date and sex were not (*Year*:  $F_{1, 99} = 9.73$ ,  $p = 0.0024$ ; *Passage Date*:  $F_{1, 99} = 0.68$ ,  $p = 0.41$ ; *Sex*:  $F_{1, 99} = 1.15$ ,  $p = 0.29$ ;  $R_c^2 = 0.10$ ). For adult sharp-shinned hawk, we found no influence of year, passage date, or sex (*Year*:  $F_{1, 98} = 0.23$ ,  $p = 0.63$ ; *Passage Date*:  $F_{1, 98} = 0.18$ ,  $p = 0.67$ ; *Sex*:  $F_{1, 98} = 0.55$ ,  $p = 0.46$ ;  $R_c^2 = 0.010$ ). For adult red-tailed hawk, we found that year and site were significant predictors of THg, whereas passage/sample collection date, sex, and the interaction between passage date and site were not (*Year*:  $F_{1, 92} = 4.26$ ,  $p = 0.042$ ; *Passage/Sample Date*:  $F_{1, 92} = 0.73$ ,  $p = 0.39$ ; *Sex*:  $F_{1, 92} = 0.92$ ,  $p = 0.34$ ; *Site*:  $F_{1, 92} = 10.99$ ,  $p = 0.0013$ ; *Passage/Sample Date\*Site*:  $F_{1, 92} = 2.52$ ,  $p = 0.12$ ;  $R_c^2 = 0.17$ ). Because site was a significant predictor of feather THg concentrations in adult red-tailed hawk, we reran the statistics just for a single site at the Marin Headlands and found that passage date (Fig. 3) was a significant predictor of THg, whereas year and sex were not (*Year*:  $F_{1, 36} = 2.10$ ,  $p = 0.16$ ; *Passage Date*:  $F_{1, 36} = 4.14$ ,  $p = 0.049$ ; *Sex*:  $F_{1, 36} = 0.001$ ,  $p = 0.97$ ;  $R_c^2 = 0.14$ ).

### Geographic results

We tested for geographical trends of THg exposure using a single species (merlin) sampled in four North American flyways where age (juvenile) and year (2009) were fixed by the experimental design. For juvenile merlin, flyway was a



**Fig. 2** Model predictions showing back log-transformed breast feather THg concentrations (µg/g dw) with partial residuals and confidence bands over a 13-year period (2002–2014) for three species in the Pacific Flyway. **a** Juvenile northern harriers showed an increase in

mean THg concentrations over the sampling period. **b** Adult sharp-shinned hawks showed no significant trend. **c** Adult red-tailed hawks showed a decrease in mean THg concentrations over the sampling period

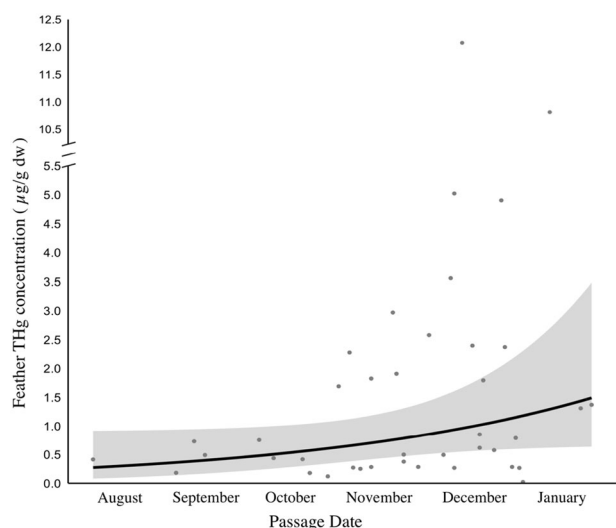
**Table 3** Total mercury (THg) concentrations (µg/g dw) in breast feathers collected from three raptor species in the Pacific Flyway over a 13-year period (2002–2014)

Year	Northern Harrier (juvenile)		Sharp-shinned Hawk (adult)		Red-tailed Hawk (adult)	
	<i>n</i> (female,male)	µg/g dw THg Mean ± SE	<i>n</i> (female,male)	µg/g dw THg Mean ± SE	<i>n</i> (female,male)	µg/g dw THg Mean ± SE
2002	11 (8,3)	0.41 ± 0.10	6 (6,0)	6.83 ± 1.94	7 (7,0)	1.14 ± 0.61
2003	2 (2,0)	0.15 ± 0.08	8 (8,0)	5.05 ± 1.29	2 (2,0)	0.49 ± 0.44
2004	11 (7,4)	0.34 ± 0.08	6 (6,0)	5.31 ± 1.51	10 (10,0)	1.50 ± 0.73
2005	–	–	11 (11,0)	7.64 ± 1.75	4 (4,0)	0.78 ± 0.51
2006	17 (11,6)	0.57 ± 0.11	11 (7,4)	5.23 ± 0.97	18 (13,5)	1.06 ± 0.32
2007	13 (6,7)	0.37 ± 0.08	13 (12,1)	7.67 ± 1.56	10 (10,0)	0.65 ± 0.31
2008	5 (2,3)	0.42 ± 0.14	12 (12,0)	4.45 ± 0.99	10 (10,0)	1.02 ± 0.50
2009	8 (2,3)	0.34 ± 0.09	12 (11,1)	4.35 ± 0.91	4 (4,0)	0.53 ± 0.36
2010	1 (0,1)	0.48	7 (7,0)	6.16 ± 1.65	3 (3,0)	0.77 ± 0.58
2011	2 (0,2)	0.55 ± 0.30	11 (11,0)	5.41 ± 1.24	5 (5,0)	0.68 ± 0.42
2012	11 (4,7)	0.63 ± 0.15	1 (1,0)	11.86	10 (10,0)	1.26 ± 0.64
2013	7 (4,3)	0.71 ± 0.21	3 (3,0)	5.13 ± 1.94	11 (11,0)	0.28 ± 0.14
2014	15 (8,7)	0.69 ± 0.13	1 (1,0)	11.79	4 (4,0)	0.14 ± 0.09

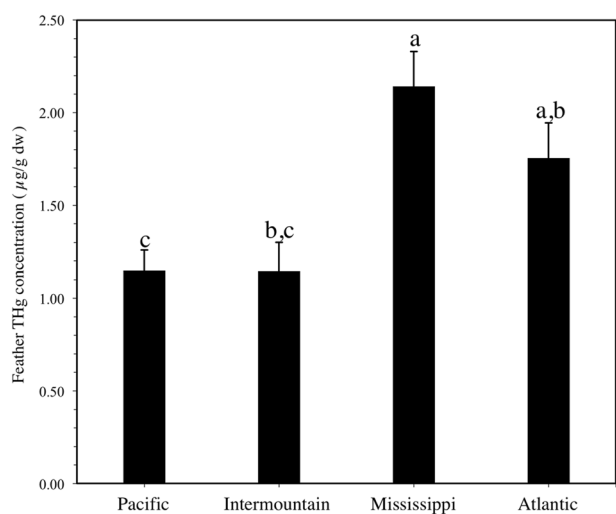
Data represents sample sizes (*n*) of juvenile northern harrier, adult sharp-shinned hawk, and adult red-tailed hawk over a 13-year period (2002–2014). For each sampling year, geometric means ± standard errors (Mean ± SE) µg/g dw of feather THg were calculated

significant predictor of THg, whereas sex was not (*Flyway*:  $F_{3, 161} = 11.09, p < 0.001$ ; *Sex*:  $F_{1, 161} = 2.57, p = 0.11$ ;  $R_c^2 = 0.18$ ). Tukey pair-wise comparisons (95% CI) revealed that merlins sampled in the Mississippi Flyway averaged higher ( $p < 0.001$ ) feather THg concentrations than merlins sampled in both the Pacific Flyway and Intermountain Flyway. Merlins sampled in the Atlantic Flyway averaged higher ( $p < 0.05$ ) feather THg concentrations than merlins sampled in the Pacific Flyway (Fig. 4; Table 4). Adults

exhibited higher feather THg concentrations than juvenile individuals within the Intermountain Flyway ( $F_{1, 27} = 22.8, p < 0.001, R_c^2 = 0.45$ ) and Mississippi Flyway ( $F_{1, 60} = 28.6, p < 0.001, R_c^2 = 0.33$ ). Adult merlin feather samples were only available from two flyways (Table 5), and as such were omitted from the full analysis, however, THg concentrations were as follows: geometric mean[95%CI]: Intermountain Flyway,  $n = 7$ ; 3.09[1.95–4.90]; Mississippi Flyway,  $n = 7$ ; 8.09[5.55–11.79].



**Fig. 3** Model prediction showing back natural-log transformed breast feather THg concentrations ( $\mu\text{g/g dw}$ ) with partial residuals and confidence bands of adult red-tailed hawk samples collected over the autumn migration season (August–January) in the Marin Headlands of California (Pacific Flyway). Each individual's sampling date was converted to Julian date and represent migration passage date through the study site



**Fig. 4** Least squares mean  $\pm$  standard error  $\mu\text{g/g dw}$  of back-transformed breast feather THg concentrations of juvenile merlin from 4 North American flyways. THg concentrations are averaged over sex and were estimated from a linear mixed-effects model with flyway and sex as fixed effects, and site as a random effect. Flyways that do not share a lower-case letter are significantly different ( $p < 0.05$ ) from each other with a 95% Tukey pair-wise comparison

## Discussion

Species, age, and flyway were significant predictors of feather THg concentrations in terrestrial-feeding raptors in western North America. All raptors sampled had measurable levels of THg in their breast feathers, consistent with the ubiquitous nature of Hg contamination; however,

notably demonstrating exposure within the terrestrial food web outside of aquatic ecosystems most commonly associated with MeHg bioaccumulation (Howie et al. 2018). Feather THg concentration levels were highly variable among species and even among individuals within a species. Hg toxicity may have different effects and consequences that vary among species (Scheuhammer et al. 2007) and feather Hg concentrations in raptors are not necessarily indicative of reproductive success or population declines (Bowerman et al. 1994; Bechard et al. 2009; Barnes et al. 2018). Therefore, the true impacts on each raptor species in this study are difficult to extrapolate from the feather THg concentrations reported, and instead may serve as a guide for future targeted research investigating contaminants levels and impacts on specific raptor populations.

Diet is the main route of MeHg exposure in vertebrates and the THg present in feathers is almost exclusively from MeHg in the blood stream at the time of feather growth. Therefore, we expected variability in feather THg concentrations among species and individuals due to differences in foraging ecology, dietary preferences, and variations in local prey composition, in addition to the variability of Hg deposited among feathers and feather tracts (Lindberg 1984; Dauwe et al. 2003; Palma et al. 2005; Roque et al. 2016). Furthermore, the feather THg concentrations in this study represent exposure on the breeding grounds where feathers were grown rather than exact location of exposure.

We found that species that presumably feed at higher trophic levels (i.e., bird specialists that consume insectivorous songbirds) had higher feather THg concentrations compared to raptors that typically consume granivorous mammals, similar to other raptor studies showing elevated Hg levels in higher trophic positions (Zolfaghari et al. 2007; Lourenço et al. 2011; Keyel 2016). Sharp-shinned hawks had the highest mean feather THg concentrations and are a bird specialist that consumes almost exclusively songbird prey (Bildstein and Meyer 2000). Increased MeHg exposure may arise through the consumption of songbirds and shorebirds that consume invertebrates with aquatic larval stages (Rimmer et al. 2010; Cristol et al. 2008). Furthermore, predatory invertebrates (e.g., spiders, dragonflies) are a common prey of songbirds that bird-eating raptors typically consume, which ultimately increases trophic level and the degree of biomagnification in raptors that consume insectivorous songbirds (Gunnarsson 2007; Cristol et al. 2008; Townsend et al. 2013). While bird-eating raptors consume a variety of insectivorous and granivorous songbird prey, most granivorous songbirds, e.g., sparrows (Emberizidae), blackbirds (Icteridae), finches (Fringillidae), songbirds, e.g., sparrows (Emberizidae), blackbirds (Icteridae), finches (Fringillidae), will still consume invertebrates



**Table 4** Model estimates for total mercury (THg) concentrations ( $\mu\text{g/g dw}$ ) in juvenile merlin breast feathers collected from four North American Flyways in 2009

Flyway	(A) Full model: averaged over sex			(B) Juvenile female			(C) Juvenile male		
	n	$\mu\text{g/g dw THg}$		n	$\mu\text{g/g dw THg}$		n	$\mu\text{g/g dw THg}$	
		LS Mean $\pm$ SE	95% CI		Mean $\pm$ SE	95% CI		Mean $\pm$ SE	95% CI
Pacific	49	1.15 $\pm$ 0.11	0.93–1.37	17	1.36 $\pm$ 0.19	0.97–1.75	32	1.00 $\pm$ 0.12	0.76–1.25
Intermountain	23	1.14 $\pm$ 0.16	0.84–1.45	11	1.40 $\pm$ 0.25	0.91–1.90	12	0.94 $\pm$ 0.19	0.56–1.31
Mississippi	56	2.14 $\pm$ 0.19	1.77–2.51	26	2.38 $\pm$ 0.28	1.83–2.93	30	1.92 $\pm$ 0.24	1.43–2.40
Atlantic	38	1.75 $\pm$ 0.19	1.38–2.13	30	1.70 $\pm$ 0.18	1.33–2.06	8	2.47 $\pm$ 0.61	1.25–3.68

(A) The full model represents least squares mean  $\pm$  standard error (LS Mean  $\pm$  SE), and 95% confidence interval (CI) calculated using a linear mixed-effects model with sample site as a random effect. Juvenile female (B) and juvenile male (C) data represent sample sizes ( $n$ ) and geometric mean  $\pm$  standard error (Mean  $\pm$  SE) and 95% CI  $\mu\text{g/g dw}$  of feather THg

**Table 5** Total mercury (THg) concentrations ( $\mu\text{g/g dw}$ ) in adult merlin breast feathers collected in the Intermountain Flyway and Mississippi Flyway in 2009

Flyway	(A) Adult female			(B) Adult male		
	n	$\mu\text{g/g dw THg}$		n	$\mu\text{g/g dw THg}$	
		Mean $\pm$ SE	95% CI		Mean $\pm$ SE	95% CI
Intermountain	3	2.29 $\pm$ 0.60	0.76–3.82	4	3.87 $\pm$ 0.87	1.63–6.12
Mississippi	4	7.98 $\pm$ 1.78	3.41–12.56	3	8.23 $\pm$ 2.12	2.78–13.68

This data is organized by sex ((A) female or (B) male) and represents sample size ( $n$ ), and geometric mean  $\pm$  standard error (Mean  $\pm$  SE) and 95% confidence interval (CI)  $\mu\text{g/g dw}$  of feather THg

part of the year, especially during the breeding season and as nestlings (Judd 1901; De Graaf et al. 1985). This pathway of contaminant exposure has also been described in the post-DDT era, where raptors consuming higher proportions of insectivorous birds had higher DDT exposure than their counterparts that consume less insectivorous prey (Keith and Gruchy 1972).

Western red-shouldered hawks (*B. l. elegans*; Hull et al. 2008a, 2008b; Dykstra et al. 2008), are not bird specialists and exhibited feather THg concentrations similar to some bird specialists in our study. Red-shouldered hawks in the west, e.g., Oregon and California, are highly associated with riparian habitats (Bloom et al. 1993; Rottenborn 2000; Dykstra et al. 2008) and their diet may include more amphibians, reptiles, and birds, compared to other *Buteos* we sampled (e.g., red-tailed hawks and broad-winged hawks) which consume predominantly small mammals. In North Carolina, liver samples collected from red-shouldered hawks also exhibited relatively high Hg concentrations compared to other birds of prey (Weir et al. 2018). It is unclear to what extent Hg exposure is affecting red-shouldered hawk populations in different regions. Further investigation is needed to assess the risks and pathways of MeHg exposure in red-shouldered hawks across their range to understand how habitat and diet are influencing the reported elevated Hg levels.

Across 13 years (2002–2014), we found no trend in feather THg concentrations for adult sharp-shinned hawks. In studies spanning similar time periods in Europe, no

temporal trends were found for northern goshawk *Accipiter gentilis*, common buzzard *Buteo buteo*, and tawny owl *Strix aluco* (Bustnes et al. 2013; Varela et al. 2016; García-Seoane et al. 2017). We found differing trends among juvenile northern harriers and adult red-tailed hawks, where THg concentrations increased and decreased, respectively. Even though both species had significant  $p$ -values related to sampling year, and mean THg concentrations doubled in juvenile northern harriers and halved in adult red-tailed hawks, there was little difference between years; therefore, these temporal trends should be interpreted with caution. Further research is required to assess whether results would be consistent with larger sample sizes and across a longer sampling period than 13-years. While a 13-year time frame may not be of sufficient length to detect long-term changes in environmental Hg exposure, the sampling period of this data set is an advancement in Hg monitoring efforts for North American raptors and provides a baseline for future comparisons as breast feather samples continue to be collected across North America.

We found increasing Hg exposure levels for adult red-tailed hawks in relation to passage date in the Marin Headlands, California, meaning that birds migrating through the migration site later in the season had higher levels of Hg exposure at time of feather growth compared to earlier migrants. Since we did not find a similar trend in the other species analyzed, this may be attributed to the differential migration timing between two genetically and geographically distinct populations of red-tailed hawks

known to migrate through the Pacific Flyway sampling site in the Marin Headlands along the California coastline, with later migrants comprised of a higher proportion from an Intermountain origin (Hull et al. 2009). These results could indicate differing Hg exposure among red-tailed hawk populations in western North America, rather than an actual effect of sampling date because breast feathers were likely grown several months prior to sampling in the breeding season. Further, but further targeted studies would be needed to confirm more precise population differences in Hg exposure.

For merlins sampled in four North American flyways, juveniles from the Mississippi Flyway averaged greater THg concentrations compared to juvenile merlins from the Pacific and Intermountain flyways. Juvenile merlins from the Atlantic Flyway also averaged greater THg concentrations compared to juveniles from the Pacific and Intermountain flyways; however, the Atlantic Flyway was only statistically greater than the Pacific Flyway. In North America, a similar east to west geographic trend was observed in both adult and juvenile common loons *Gavia immer* (Evers et al. 1998). Merlins breed across the boreal forest and Pacific Northwest, so juvenile feather THg concentrations may provide an index of Hg exposure for this broad geographic region. Our analysis consisted of one sampling site within the Pacific, Mississippi, and Atlantic flyways. However, considering that feather THg concentrations represent Hg exposure on the breeding grounds and distinct migratory paths vary among raptor populations (Goodrich and Smith 2008) and are associated with morphological and genetic differences in multiple raptor species (Hull and Girman 2005; Hull et al. 2008a, 2008b; Pitzer et al. 2008; Preston and Beane 2009), our results may indicate differential exposure among merlins breeding in distinct regions across their breeding range.

Generally, Hg concentrations in birds are higher in males than in females (Ackerman et al. 2007, 2008; Robinson et al. 2011); however, we found no significant difference in feather THg concentrations between sexes for raptor species. Raptors exhibit varying degrees of reverse sexual size dimorphism, where females are larger than males. For example, the difference in size between sharp-shinned hawk females and males is greater than it is for red-tailed hawks (Snyder and Wiley 1976; Bildstein 1992). In juveniles, we may expect females to deposit lower Hg concentrations in growing feathers compared to males due to dilution effect, however, we did not find a statistical difference among juveniles in our study. Furthermore, feather Hg concentrations in adult females may appear lower than actual exposure if feather molt occurred after egg laying as Hg can be excreted through egg production (Lewis and Furness 1993; Ackerman et al. 2016b). Similarly, studies investigating Hg concentrations in internal tissues for other raptor species

also did not find a significant difference among sexes, for example laggar falcons *Falco jugger*, northern goshawk, common buzzard, and tawny owl (Movalli 2000; Castro et al. 2011). It is possible that uneven and low sample size among sexes within a species may have contributed to this result in our study; however, due to the variation in sexual size dimorphism among species, investigating the interaction between age and sex may help us understand how sexual size dimorphism may contribute to differential Hg exposure between species. Ultimately, further research is needed to understand the differences between female and male Hg deposition in breast feathers and how it relates to potential resource partitioning and physiology.

Lastly, age was a significant predictor of feather THg concentrations in our study, with adults averaging greater feather THg concentrations than juveniles. This result was expected because Hg deposited in juvenile breast feathers represent exposure from prey provisioned while in the nest as feather tracts are growing and juveniles are rapidly increasing in size and diluting their Hg concentrations (Ackerman et al. 2011), whereas Hg deposited in adult breast feathers represent exposure at the time of body feather molt (Garcia-Seoane et al. 2017; Barnes and Gerstenberger 2015; Evers et al. 2005). Due to the differences in Hg feather deposition between juveniles and adult raptors, when using THg concentrations in breast feathers as an index of Hg exposure among raptor species, comparisons may be more accurate if analyzed within a single age class.

## Conclusion

At the large geographic scale of North American flyways, interspecific and geographic trends were detected among terrestrial feeding raptor species. Feather THg concentrations varied among raptor species and age groups; however, Hg exposure may be influenced by region and was generally higher in species that specialize in feeding on birds, with the exception of red-shouldered hawks which had relatively similar feather THg concentrations to bird specialists.

Additionally, analyzing samples from cosmopolitan raptor species, such as merlins, may be informative for identifying broad-scale geographic trends in Hg exposure, and analyzing terrestrial feeding raptors that consume a high proportion of insectivorous songbirds, such as sharp-shinned hawks, may be informative for monitoring MeHg biomagnification in terrestrial food webs. Within migration corridors where distinct populations may vary temporally as migrants move through a monitoring site, e.g. red-tailed hawk, changes in feather THg concentrations across passage dates may indicate differential Hg exposure among populations from distinct regions.

Lastly, utilizing archived feather samples collected by raptor migration monitoring stations across the continent allowed us to look at Hg exposure for many species representative of different feeding guilds across space and time in a minimally invasive way. Therefore, breast feathers collected by networks of migration monitoring stations may have a role in biomonitoring contaminants in North America and around the world.

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## Compliance with ethical standards

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

**Ethical approval** All national and institutional guidelines for the care and use of animals were followed. All raptor handling and feather collections were in compliance with the Golden Gate Raptor Observatory standards as described in their Institutional Animal Care and Use Committee protocols. This article does not contain any studies with human participants performed by any of the authors.

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