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## A BROADSCALE ASSESSMENT OF MERCURY CONTAMINATION IN PEREGRINE FALCONS ACROSS THE NORTHERN LATITUDES OF NORTH AMERICA

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**ABSTRACT.**—We document concentrations of total mercury (THg) in feathers of Peregrine Falcons (*Falco peregrinus*; hereafter peregrines) collected during autumn migration at South Padre Island, Texas, and Assateague Island, Maryland, from 2009–2015. We detected THg in all sampled fourth primary (p4; range = 0.44–37.46 µg/g) and axillary feathers (range = 0.09–62.68 µg/g). We found no significant difference in THg concentrations between hatch-year (HY) peregrines by study site. Mean THg concentrations were greater in both feather types of after-hatch-year peregrines than of HY peregrines, but concentrations in p4 feathers of second-year peregrines (mean = 14.9 µg/g) were significantly greater than those of after-second-year individuals (mean = 8.5 µg/g). Pooling samples from HY birds across both sites and all years, we found no significant differences between the concentrations in the axillaries of females (mean = 2.4 µg/g) vs. males (mean = 2.2 µg/g), nor between the two feather types. The concentration associated with toxic effects in peregrines is unknown; however, peregrines have recently experienced broad population expansion across the presumed breeding area of the birds we sampled, and the THg concentrations we measured were lower than those in an apparently healthy breeding population in the southwestern USA. We documented widespread THg exposure in peregrines migrating from the northern latitudes of North America, but additional research is needed to assess trends of mercury exposure in the face of increasing global anthropogenic release of mercury into the environment and the release of long-term sequestered mercury in melting permafrost because of climate change.

**KEY WORDS:** *Peregrine Falcon*; *Falco peregrinus*; *contaminant testing*; *feathers*; *Maryland*; *mercury*; *migration*; *Texas*; *toxicology*.

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ESTUDIO A GRAN ESCALA DE LA CONTAMINACIÓN CON MERCURIO EN *FALCO PEREGRINUS* EN LATITUDES SEPTENTRIONALES DE AMÉRICA DEL NORTE.

RESUMEN.—Evaluamos la concentración de mercurio total (THg) en plumas de *Falco peregrinus* recolectadas durante la migración otoñal en las islas South Padre, estado de Texas, y Assateague, estado de Maryland, entre los años 2009 y 2015. Detectamos THg en todas las plumas primarias p4 (rango = 0.44–37.46 µg/g) y axilares muestreadas (rango = 0.09–62.68 µg/g). Para ambos sitios, no se encontraron diferencias significativas en las concentraciones de THg entre los individuos del primer año. Las concentraciones promedio de THg fueron mayores, en ambos tipos de plumas, en los individuos mayores al primer año de eclosión que en individuos del primer año. Sin embargo, las concentraciones en las plumas p4 de los individuos del segundo año (media = 14.9 µg/g) fueron significativamente más grandes que en las de los individuos mayores al segundo año (media = 8.5 µg/g). Agrupando las muestras de los individuos del primer año por sitio de estudio como también por años, no encontramos diferencias significativas en las concentraciones de mercurio halladas en plumas axilares de hembras (media = 2.4 µg/g) y machos (media = 2.2 µg/g), ni entre el tipo de plumas (primarias y axilares). En *F. peregrinus* se desconoce el nivel de concentración de mercurio asociado con efectos tóxicos. Sin embargo, *F. peregrinus* ha experimentado recientemente una amplia expansión de sus poblaciones a lo largo de las áreas estimadas de cría de las aves muestreadas, con concentraciones de THg menores que las concentraciones observadas en una población reproductora aparentemente saludable del sudoeste de EEUU. Finalmente, registramos una exposición generalizada a THg en los individuos de *F. peregrinus* que migran desde las latitudes septentrionales de América del Norte. Se necesitan mayores estudios para poder evaluar las tendencias en la exposición a mercurio en el contexto del aumento global en la liberación antropogénica de este elemento en el ambiente y también, como consecuencia del cambio climático, del mercurio liberado desde el permafrost.

[Traducción del equipo editorial]

Raptors and various piscivorous birds, as top avian predators, can be vulnerable to persistent environmental contaminants from biomagnification over progressive trophic levels and, as such, they can be good indicators of environmental pollution (Evers et al. 1998, Burger and Gochfeld 2004, Lodenius and Solonen 2013). One heavy metal pollutant, mercury (Hg), occurs naturally in the environment, but has increased substantially from anthropogenic causes since the industrial revolution (Swain et al. 1992, Arctic Monitoring and Assessment Programme [AMAP 2011], United Nations Environment Programme [UNEP 2013]). Although elevated Hg levels are typically greatest in aquatic systems (AMAP 2011, Ackerman et al. 2016), Hg in food webs of terrestrial systems also poses a risk to biotic systems (Cristol et al. 2008, Townsend et al. 2013, Keller et al. 2014). Many birds have been used as indicators of environmental Hg contamination, such as Common Loons (*Gavia immer*; Evers et al. 1998), marine birds (Bond and Diamond 2009), wading birds (Frederick et al. 2002), and Bald Eagles (*Haliaeetus leucocephalus*; Bowerman et al. 2002). However, research on these species, and much of the avian contaminant research as a whole, has largely focused on aquatic systems generally with restricted geographical distributions.

Atmospheric deposition since the beginning of the industrial revolution in the mid-1800s has been a major driver of elevated global Hg levels, specifically in the boreal, subarctic, and Arctic regions (hereafter northern latitudes; AMAP 2011). Evidence in recent decades has shown a stable or declining trend of atmospheric deposition of Hg in North American northern latitudes (AMAP 2011), yet recent climate change trends appear to be driving increased exposure risk of biota due to mobilization of Hg from melting permafrost coupled with boreal riverine transport (Schuster et al. 2011) and increased wildfire frequency (Matz et al. 2011). Studies in the northern latitudes have demonstrated elevated Hg concentrations in taxa as diverse as freshwater fish (Carrie et al. 2010), beluga whales (*Delphinapterus leucas*; Lockhart et al. 2005), and polar bears (*Ursus maritimus*; Dietz et al. 2006a), with increases in recent decades shown in a 130-yr time-series analysis of Ivory Gulls (*Pagophila eburnea*; Bond et al. 2015) and a 150-yr study of Greenlandic raptors (Dietz et al. 2006b). Dietz et al. (2006b) analyzed feathers from Peregrine Falcons (*Falco peregrinus*, hereafter peregrines) from West Greenland from 1859–2001 and detected an increasing trend of Hg concentrations in adults after 1975, but their analysis was limited by small sample size.

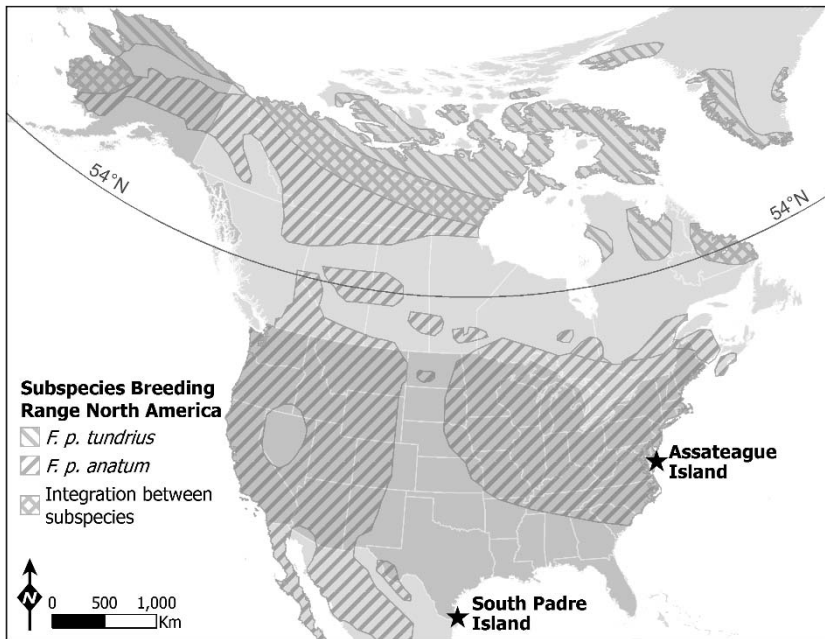


Figure 1. Map of the North American breeding ranges of the Peregrine Falcon subspecies, *Falco peregrinus tundrius* and *F. p. anatum*, and their area of overlap. Stars indicate our autumn migration study sites, South Padre Island, Texas (2009, 2013–2015) and Assateague Island, Maryland (2009, 2015). Breeding ranges were redrawn from White et al. (2013).

Peregrines have the potential to be a particularly useful biomonitor because they are globally distributed, nest in proximity to a diverse assortment of aquatic and terrestrial habitat types (e.g., freshwater rivers and lakes, marine systems, estuaries, wetlands, arctic tundra, temperate forests, and open desert), are long-lived with high breeding-site fidelity, and have an extremely diverse avian diet (White et al. 2013). The migratory nature of many peregrine populations, and the extensive and highly variable migration patterns of their various avian prey species are important factors that must be considered when assessing geographic and temporal Hg exposure. Earlier peregrine contaminant studies assessed organochlorine pollutants (Henny et al. 1982, Henny et al. 2009, Franke et al. 2010) and other chemical pesticides (Newton et al. 1989, Peakall et al. 1990), petroleum exposure (Seegar et al. 2015), trace metals and various environmental contaminants (Parrish et al. 1983, Ambrose et al. 2000), and Hg (Lindberg and Mearns 1982, Dietz et al. 2006b, Barnes and Gerstenberger 2015). Mercury concentrations in peregrine feathers are correlated with Hg levels in their prey (Lindberg and Odsjö 1983, Barnes and Gerstenberger 2015); however, the

threshold toxicity levels that impair reproduction, health, and survival have not been established in this species.

After assessing 11 microsatellite loci sampled from migrating peregrines and samples collected from throughout their northern-latitude breeding grounds, Johnson et al. (2010) determined that peregrines migrating through Padre Island, Texas, derived largely from breeding areas across northern Alaska, Canada, and western Greenland. Franke (2016) compiled banding and encounter records from hatch-year (HY) peregrines (*F. p. tundrius*, and northern-breeding *F. p. anatum* from the taiga) banded from 1970–2010 in Alaska north of 60°N latitude, Canada north of 54°N latitude, and the west coast of Greenland (Fig. 1). His assessment of band encounters concurred with an earlier synthesis of peregrine banding and encounter records from 1955–1985 by Yates et al. (1988), who found that the northern-latitude peregrines largely partitioned themselves during autumn migration into two largely distinct, population segments. They differentiated a “western” population originating from Alaska, Yukon, and Northwest Territories and mostly passing through South Padre Island, Texas (SPI; a

focal point for the Gulf of Mexico migration route), from an “eastern” population originating from Nunavut, Quebec, and Greenland and mostly migrating through Assateague Island, Maryland (AI; a focal point for the North American East Coast migration route; Fig. 1).

These prior studies, as well as satellite tracking results (Fuller et al. 1998, McGrady et al. 2002), all indicate that the majority of peregrines encountered at our two study sites originate from Arctic and boreal natal and breeding areas, so we sought to use feathers collected from migrating peregrines in autumn at SPI and AI to inform us remotely of contemporary Hg exposure at northern latitudes during the breeding season (i.e., the time and place where feathers were grown). We initially drew from archived axillary feathers collected in 2009 from the two study sites, and then also sampled fourth primary flight feathers (p4) from 2013–2015 in an effort to refine our knowledge of spatial and temporal Hg concentrations. Additional objectives were to assess differences in Hg exposure by age class and sex, compare Hg concentrations between feather types, and look for changes in Hg levels over time.

#### METHODS

**Study Area.** We analyzed peregrine feathers collected during autumn migrations in 2009, and 2013–2015 on SPI and in 2009 and 2015 on AI (Fig. 1). Our trapping area on SPI (26°18.8'N, 97°12.4'W) encompassed approximately 40 km of the northern end of the barrier island off the coast of Texas in the Gulf of Mexico, the majority of which was administered by Laguna Atascosa National Wildlife Refuge. Our trapping area on AI (38°1.7'N, 75°14.5'W) stretched approximately 36 km along a barrier island off the Atlantic coast of Maryland, within the Assateague Island National Seashore. The habitat on SPI consisted primarily of un-vegetated wind-tidal flats on the western (leeward) side of the island, freshwater wetlands in inter-dune swales and wash-over channels, and coastal beaches to the east. The habitat on AI differed from SPI in the elimination of wind-tidal flats west of the dunes in recent decades resulting from anthropogenic activities; otherwise physical habitat characteristics of the two islands were similar.

**Feather Collection.** The analysis of Hg in feathers is a noninvasive approach that allows for resampling of individuals over time, and allows for testing archived samples and museum specimens for long-

term trend analysis (Berg et al. 1966, Frederick et al. 2004, Dietz et al. 2006b). This is effective because Hg is sequestered in feathers during feather growth (Appelquist et al. 1984), it persists over time (Berg et al. 1966), and its concentration is correlated with dietary Hg consumption (Fimreite and Karstad 1971, Lewis and Furness 1991, Spalding et al. 2000). When conducting feather Hg analyses, it is important to assess standardized feathers or feather types based on timing of feather replacement within the molt cycle (Furness et al. 1986, Braune 1987, Dauwe et al. 2003) because most birds undergo predictable molt cycles and feather molt is the major elimination pathway for Hg (Honda et al. 1986, Lewis and Furness 1991, Lewis et al. 1993).

We trapped migrating peregrines daily at each study site during all daylight hours (approximately 0730–1900 H CST on SPI, and 0700–1900 H EST on AI) from 25 September to 25 October each year depending on favorable weather (i.e., generally wind <25 km/hr and minimal precipitation). We used a roving trapping approach, with each trapper using an all-terrain vehicle (ATV) or other four-wheel-drive vehicle to opportunistically cover the trapping area and to position themselves for trapping migrating peregrines as they were encountered. Generally, two ATVs operated simultaneously on SPI, with trapping confined to un-vegetated wind-tidal flats west of unstable dunes. A single trapping team in a four-wheel-drive vehicle operated at AI, where most trapping occurred on coastal beaches. We used harnessed Rock Pigeons (*Columba livia*) attached to a weighted tether and fitted with monofilament nooses to capture peregrines (Bloom et al. 2007). We classified peregrine age classes based on plumage (Pyle 2008), such that the plumage of HY peregrines was entirely juvenile feathers, while second-year (SY) peregrines exhibited a mix of juvenile and second basic feathers, and after-second-year (ASY) peregrines were entirely definitive basic plumage. We classified captured peregrines as after-hatch-year (AHY) when we could not distinguish between SY and ASY, and for pooled age-class comparisons. We sexed individuals using published wing-chord lengths (Pyle 2008). We banded each peregrine with a standard locking US Geological Survey (USGS) aluminum band unless it had previously been banded, in which case we contacted the USGS Bird Banding Laboratory for banding location and date to determine natal or breeding area if possible. This information allowed us to make broadscale assessments of Hg contamination of

peregrines across northern latitudes of North America even though our sampling occurred at only two migration sites.

Using clean stainless steel scissors, we sampled axillaries by cutting near the base of the feather vane excluding the calamus. When sampling p4, we removed 1.5–2.0 cm of the distal end of each feather for analysis. Adhering to a set length of standardized feathers allowed us to account for variable growth rates in various feathers (Bortolotti 2010). We stored all feather samples in acid-free paper coin envelopes at room temperature prior to analysis.

In 2009, we randomly selected archived axillary feathers from 30 HY peregrines from both study sites and 15 AHY peregrines from SPI to determine whether peregrine feathers contained detectable levels of Hg during autumn migration. In our following investigations at SPI, we sampled one axillary feather from each HY peregrine and an axillary and p4 feather from AHY peregrines that were captured during 2013–2015. Not encountering large numbers of older age classes at AI, we sampled an axillary from each HY peregrine in 2015 after our initial 2009 assessment.

**Feather Analysis.** Total Hg (THg) includes all possible forms of mercury (e.g., elemental Hg, organomercury, inorganic Hg, methylmercury, etc.). This is important from a toxicological standpoint because different forms of Hg vary widely in their toxicity (i.e., elemental and inorganic Hg have low toxicity, whereas methylmercury is highly toxic). Virtually all of the Hg in feathers is methylmercury, so THg is a surrogate of the more toxic organic methylated form (Bond and Diamond 2009).

To assess THg in feathers, we used an AMA 254 atomic absorption spectrometer (method detection limit = 2.5 ng/g [ppb]; Leco Corporation, St. Joseph, MI, USA) at the environmental and occupational health laboratory at the University of Nevada, Las Vegas. We present our results as THg on a fresh weight (fw) basis in  $\mu\text{g/g}$  (equivalent to ppm). We analyzed each sampled axillary feather in its entirety, and analyzed 1.5 cm of the distal end of each p4. Feather samples averaged 6.2 mg for axillaries ( $n = 521$ ), and 8.4 mg for p4 ( $n = 227$ ). After we scrubbed feathers to remove all surface debris prior to analysis, they underwent thermal decomposition (750°C for 320 sec) and were carried by ultra-pure  $\text{O}_2$  to a gold-plated amalgamator using a 253.65-nm wavelength light to determine THg concentrations in each sample. We ran quality control tests every 10 samples, using a method blank and one or two

samples of standard certified reference material (CRM; CRM 1633b and CRM 2709; National Institute of Standards and Technology, Gaithersburg, MD, USA), to verify calibration. Accepted CRM recoveries ranged from 88–111% of the certified values of THg (mean =  $100.5 \pm 5.9\%$ ,  $n = 104$ ).

**Statistical Analysis.** We conducted five separate analyses of concentrations of THg in peregrines. We conducted an overview of all birds sampled, across-individual analyses of THg in axillary feathers, across-individual analyses of THg in p4 feathers, an across-feather type analysis within individuals, and an assessment of known-origin peregrines.

To conduct our overview analyses of all birds sampled, we assessed variation in mean THg concentrations in axillary and p4 feathers, by using an analysis of variance (ANOVA) mixed effects model with age, feather type, and their interaction as fixed effects (Pinheiro and Bates 2000). We log-transformed THg concentrations prior to analysis to meet model assumptions of normal distribution and homogeneity of variance, and used pairwise differences based on a Tukey Post-hoc test to explore differences in means. We restricted our analysis to females from 2013 because sample sizes were unbalanced between years, it was the only year we collected both feather types from all age classes, and we were only able to sample females of all age classes (i.e., insufficient male sample size).

To conduct our across-individual analyses of THg in axillary feathers, we used a mixed effects model among all years at SPI, using an unequal variance model to estimate effects (Pinheiro and Bates 2000). The model included sex as a fixed effect, year as a random effect, and the interaction between year and sex as a random effect. Random effects were tested using the likelihood ratio test, and those effects which were not significant were removed from the model sequentially. To further explore differences in THg concentrations in axillaries by sex, while assessing potential differences between study site, we also used a mixed effects model assuming equal variances between sexes in HY peregrines at SPI and AI in 2009 and 2015, with sex as a fixed effect, and year and site as random effects. We restricted this analysis to the subset of HY peregrines sampled in 2009 and 2015 because we were limited to sampling those years at AI. We were not able to trap a sufficient number of older males to test differences of THg concentrations between sexes of AHY peregrines.

To conduct our across-feather type analysis within individuals, we used paired sample *t*-tests to assess differences of THg concentrations within individuals with >1 feather sampled. We performed a Pearson's coefficient correlation to further investigate the relationship between axillary and p4 feathers sampled within individuals. We did not transform THg concentrations prior to analyzing differences within individuals, because differences between feathers were normally distributed.

To conduct our assessment of known-origin peregrines, we obtained natal or breeding locations from the USGS Bird Banding Laboratory for seven banded peregrines we captured at SPI that were originally banded in northern North America, and one HY individual initially captured at SPI and later recaptured on her breeding territory. Our small sample size did not allow for a statistical analysis. We did not know the specific natal or breeding areas for the other sampled peregrines, but previous research indicated nearly all peregrines migrating through our study sites originated from Arctic and boreal natal and breeding areas of North America and Greenland (Yates et al. 1988, Johnson et al. 2010, Franke 2016), which largely overlapped locations for the previously banded individuals we trapped.

Unless otherwise noted, we log-transformed THg concentrations prior to analysis to meet model assumptions of normal distribution and homogeneity of variance. We back-transformed reported values, calculated arithmetic means  $\pm$  SE, and considered results significant at  $P \leq 0.05$ . All models were run in R3.3.0 (R Development Core Team 2016) using the "nlme" package (Pinheiro et al. 2016) and the "predictmeans" package (Dongwen et al. 2014).

## RESULTS

We analyzed feathers from 518 peregrines; 464 sampled at SPI during autumn migrations (45 in 2009, 190 in 2013, 92 in 2014, and 137 in 2015), and 29 and 25 HY peregrines sampled at AI in 2009 and 2015, respectively. In total, we analyzed feathers from 110 male and 281 female HY peregrines, and 3 male and 124 female AHY peregrines. All feather samples contained detectable concentrations of THg (range = 0.09–62.68  $\mu\text{g/g}$ ; Table 1). Mean THg concentrations pooled by year and sex in axillary and p4 feathers of HY peregrines were similar, but both feather types contained greater concentrations of total Hg in AHY (SY and ASY) than in HY peregrines.

Table 1. Total mercury concentrations ( $\mu\text{g/g}$  fresh weight) detected in axillary and fourth primary (p4) feathers of migrating Peregrine Falcons during autumn migrations on South Padre Island, Texas (2009, and 2013–2015), and Assateague Island, Maryland (2009, 2015). Age class (i.e., hatch-year, second-year, after-second-year) was determined by plumage from Pyle (2008). Arithmetic means are presented, and year and sex are pooled for each age class/feather type category.

AGE CLASS AND FEATHER TYPE	<i>n</i>	MERCURY CONCENTRATION ( $\mu\text{g/g}$ )	
		MEAN $\pm$ SE	RANGE
Hatch-year			
Axillary	328	2.92 $\pm$ 0.11	0.09–15.58
Axillary <sup>a</sup>	54	2.95 $\pm$ 0.24	0.54–7.78
p4	120	2.72 $\pm$ 0.17	0.44–9.35
Second-year			
Axillary	36	5.17 $\pm$ 0.91	0.64–25.83
p4	26	14.9 $\pm$ 1.32	5.0–25.58
After-second-year			
Axillary	71	7.03 $\pm$ 1.11	0.79–62.68
p4	67	8.45 $\pm$ 0.66	2.11–37.46

<sup>a</sup> Collected from Assateague Island; all others were collected from South Padre Island.

In an analysis of the overall variation in mean THg concentrations by feather type and age class, a mixed effects ANOVA model of females sampled at SPI in 2013 indicated significant differences by age class ( $F_{1,3} = 1197.1$ ,  $P = <0.001$ ), feather type ( $F = 65.7$ ,  $P = <0.001$ ), and the interaction between age class and feather type ( $F_{1,3} = 28.0$ ,  $P = <0.001$ ; Fig. 2). Our model indicated THg concentrations in p4 feathers were significantly greater than those in axillaries in each age class except HY (Fig. 2). All other age classes contained greater concentrations of THg in p4 feathers than HY females in our model, with SY individuals exceeding all others except AHY individuals (those that were not distinguished between SY and ASY age classes; Fig. 2).

**Axillary Feathers.** In a mixed effects model analyzing across years, we found no significant difference in mean THg concentrations in axillary feathers by year ( $P = 0.21$ ) or sex ( $P = 0.15$ ) in HY peregrines at SPI (female =  $2.4 \pm 0.15$   $\mu\text{g/g}$ ,  $n = 248$ ; male =  $2.2 \pm 0.16$   $\mu\text{g/g}$ ,  $n = 78$ ), nor in an interaction between year and sex ( $P \leq 1.0$ ). Comparing between sites, we found no significant difference between THg concentrations in axillaries of HY peregrines sampled at SPI ( $2.98 \pm 0.16$   $\mu\text{g/g}$ ,  $n = 139$ ) and AI ( $2.95 \pm 0.24$   $\mu\text{g/g}$ ,  $n = 54$ ) in 2009 and 2015 ( $t_{191} = 0.081$ ,  $P = 0.936$ ). There also was no

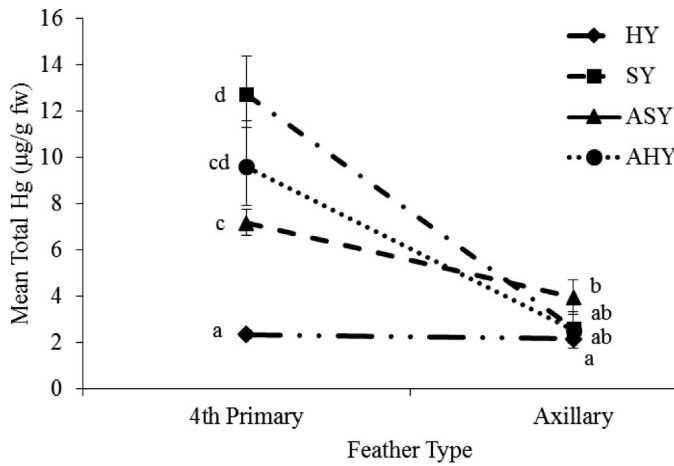


Figure 2. Concentrations of mean total mercury (THg; µg/g fresh weight) by age class detected in fourth primary and axillary feathers collected from migrating female Peregrine Falcons captured at South Padre Island, Texas, in autumn 2013. Back-transformed means with standard error bars of THg concentrations are shown from our mixed-effects ANOVA model including feather type and age as fixed effects. Letters next to each symbol indicate significant difference among means, determined using Tukey post-hoc differences <math>P < 0.05</math>. Fourth primary feather sample size varied: 82 hatch-year (HY), 15 second-year (SY), 40 after-second-year (ASY), and 11 after-hatch-year (AHY). Axillary feather sample size also varied: 78 hatch-year (HY), 15 second-year (SY), 39 after-second-year (ASY), and 11 after-hatch-year (AHY). Only those individuals older than HY and not identified more precisely by plumage were categorized as AHY.

significant difference of THg concentrations in axillaries of HY peregrines between females (mean =  $2.32 \pm 0.13$  µg/g;  $n = 132$ ) and males (mean =  $2.54 \pm 0.21$  µg/g;  $n = 61$ ;  $F_{1,188} = 0.26$ ,  $P = 0.61$ ) in a subset of HY birds from SPI and AI in 2009 and 2015. An ANOVA mixed effects model, which was restricted to feathers collected from females at SPI in 2013, indicated overlap among age classes of mean concentrations of THg in axillaries except for ASY females, which contained significantly greater concentrations than HY females (Fig. 2).

**Primary Feathers.** Arithmetic mean THg concentrations in p4 feathers ranged from  $2.74 \pm 0.17$  µg/g in 2013 HY peregrines ( $n = 117$ ) to  $17.25 \pm 2.82$  µg/g in 2015 SY peregrines ( $n = 8$ ; Fig. 3). An ANOVA mixed effects model, which was restricted to feathers collected from females at SPI in 2013, indicated significant differences in mean concentrations of THg in p4 feathers between known age classes such that  $HY < ASY < SY$  (Fig. 2).

**Sampling within Individuals.** Among those HY individuals with both feather types sampled ( $n = 111$ ), axillaries (mean =  $2.54 \pm 0.16$  µg/g) had significantly less THg than p4 (mean =  $2.73 \pm 0.18$  µg/g;  $t_{110} = -2.49$ ,  $P = 0.014$ ) feathers. There was a strong positive correlation between concentrations in axillary and p4 feathers in HY individuals with

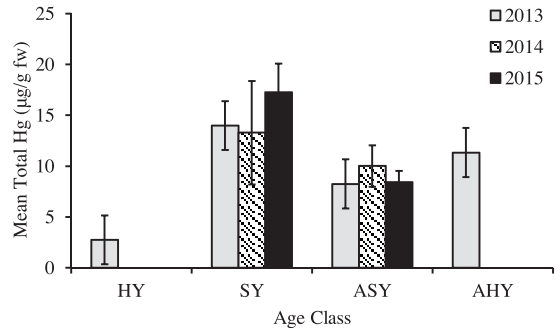


Figure 3. Concentrations of mean total mercury (µg/g fresh weight) detected in fourth primary feathers collected from migrating Peregrine Falcons captured at South Padre Island, Texas, during autumn migrations in 2013–2015. Arithmetic means are presented with standard error bars. Years and age class categories are shown, with 117 hatch-year, 15 second-year (SY), 44 after-second-year (ASY), and 11 after-hatch-year (AHY) peregrines sampled in 2013; 3 SY and 6 ASY sampled in 2014; and 8 SY and 17 ASY individuals sampled in 2015. Only those individuals older than HY and not identified more precisely by plumage were categorized as AHY.



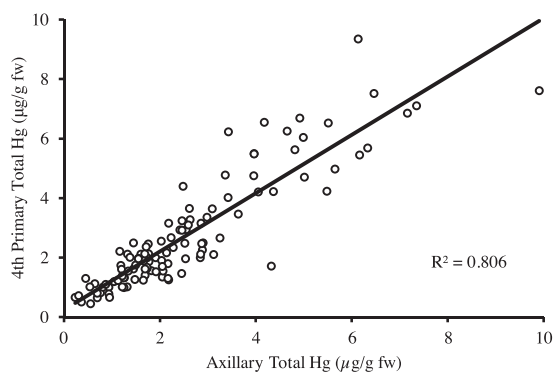


Figure 4. Scatter plot of total mercury (THg;  $\mu\text{g/g}$  fresh weight) concentrations detected in hatch-year Peregrine Falcons with axillary and fourth primary feather samples collected at the time of capture ( $n = 111$ ). Migrating peregrines were sampled during the 2013 autumn migration at South Padre Island, Texas. Data have been fitted with a linear trend line to show the relationship of THg exposure between the two feather types within individual peregrines.

both feather types sampled ( $r = 0.898$ ,  $n = 111$ ,  $P < 0.001$ ; Fig. 4). However, this predictive relationship between feather types was not apparent in older age classes. Second-year individuals with both feathers sampled ( $n = 24$ ) contained far lower THg concentrations in axillary (mean =  $5.21 \pm 0.97 \mu\text{g/g}$ ) than in p4 (mean =  $14.6 \pm 1.35 \mu\text{g/g}$ ;  $t_{23} = -5.934$ ,  $P < 0.001$ ) feathers. After-second-year individuals with both feather types sampled ( $n = 61$ ) also contained less THg in axillary feathers on average (mean =  $6.97 \pm 1.25 \mu\text{g/g}$ ) than in p4 (mean =  $8.4 \pm 0.7 \mu\text{g/g}$ ) feathers, but the difference was not significant when paired within the individual ( $t_{60} = -1.548$ ,  $P = 0.127$ ).

**Peregrines of Known Origin.** We determined natal or breeding areas for seven previously banded peregrines captured during migration on SPI, and one initially captured on SPI and later captured on her breeding territory. The peregrines with known natal areas originated from Rankin Inlet, Nunavut, Canada ( $n = 4$ ;  $62^{\circ}48.4'N$ ,  $92^{\circ}5.2'W$ ), Edmonton, Alberta, Canada ( $n = 1$ ;  $53^{\circ}32.6'N$ ,  $113^{\circ}29.4'W$ ), Red Deer, Alberta, Canada ( $n = 1$ ;  $52^{\circ}16.0'N$ ,  $113^{\circ}48.7'W$ ), and Grand Rapids, North Dakota, USA ( $n = 1$ ;  $46^{\circ}26.6'N$ ,  $98^{\circ}22.2'W$ ). Four peregrines originating from Rankin Inlet were trapped at SPI as HY individuals on their first autumn migration with a mean THg concentration in axillaries of  $3.53 \mu\text{g/g}$  (HY females =  $1.92$  and  $3.23 \mu\text{g/g}$ ; HY male =  $2.9$  and  $6.05 \mu\text{g/g}$ ), and an additional peregrine banded as a

HY on migration at SPI subsequently entered the Rankin Inlet breeding population (A. Franke pers. comm.) and was recaptured during its fourth year at SPI (p4 =  $9.84 \mu\text{g/g}$ ; axillary =  $1.63 \mu\text{g/g}$ ). Total Hg concentrations in axillaries from HY females captured at SPI and originating from Red Deer, Alberta ( $1.73 \mu\text{g/g}$ ), Grand Rapids, North Dakota ( $1.35 \mu\text{g/g}$ ), and from an Edmonton, Alberta hacking program ( $0.09 \mu\text{g/g}$ ) were all lower than those in HY females overall (i.e., female mean =  $2.4 \pm 0.15 \mu\text{g/g}$ ).

#### DISCUSSION

Our study provides a contemporary (2009–2015) population-level assessment of THg exposure in peregrines with natal and breeding areas across the vast area encompassing northern Alaska, northern Canada, and western Greenland. We base likely natal and breeding areas of our sampled peregrines on over 40 yr of migration study (Seegar et al. 2016), including results from genetic analyses (Johnson et al. 2010), band encounters (Yates et al. 1988, Franke 2016), and satellite tracking (Fuller et al. 1998, McGrady et al. 2002). Results of our initial assessment of archived axillary feathers collected during the autumn 2009 migration indicated all AHY peregrines sampled at SPI and all HY peregrines sampled at SPI or AI contained detectable concentrations of THg. Our ensuing investigations of axillaries from both age classes at SPI during the autumn migrations of 2013–2015 and HY peregrines from AI in 2015 confirmed widespread THg exposure, and we did not detect significant differences by year or study site. Our analysis of p4 feathers from peregrines captured at SPI during 2013–2015 further confirmed the widespread exposure we detected in axillaries.

Total Hg burdens in HY peregrine feathers are an index of dietary Hg exposure during the nestling period while the peregrines are on their northern North American natal areas. As expected, THg concentrations in the axillary and p4 feathers of HY peregrines were strongly correlated, and did not differ substantially, presumably because the juvenal plumage is grown concurrently prior to fledging. As expected, because young birds are provisioned with the same prey regardless of sex, we did not detect significant differences in THg concentrations by sex in either axillary or p4 feathers of HY peregrines. Similarly, an assessment of HY peregrines migrating and wintering along coastal Washington did not detect a significant difference in THg exposure

between sexes (Barnes et al. 2018), nor did Barnes and Gerstenberger (2015) detect significant differences between siblings of different sexes within peregrine broods in southern Nevada.

Short-term, site-specific exposure of THg from diet at the time of feather growth is reflected in feathers of juvenile birds (Becker et al. 1994, Ackerman et al. 2011), while THg in feathers of adults reflects Hg uptake since the previous molt and concentrations in blood at the time of feather growth (Evers et al. 2005, Perkins et al. 2016). This indicates THg concentrations found in feathers from the two age classes should differ on temporal and spatial scales, because migratory individuals accumulate Hg from various locations throughout the previous year (e.g., migration, staging, and wintering areas). This suggests the significantly greater THg concentrations we detected in AHY compared to HY peregrines may be driven by a longer timeframe of exposure in addition to differing geographic exposure.

Mean THg concentrations in p4s of SY individuals (mean = 14.9  $\mu\text{g/g}$ ) were over five times greater than we detected in HY (mean = 2.72  $\mu\text{g/g}$ ) and almost twice as great as ASY individuals (mean = 8.45  $\mu\text{g/g}$ ). All SY and all but three ASY birds were female. The decline we detected in THg concentrations in ASY compared to SY peregrines was unanticipated, because we expected to detect increases in individuals as they age due to bioaccumulation. One possible explanation suggests that because female peregrines often begin breeding by their third year (Ratcliffe 1993), the reduced THg concentrations we detected in older females may be due to the additional annual elimination pathway of eggs (Lewis et al. 1993); however, we were not able to collect corresponding data on adult males. Similarly, an analysis of HY female peregrines recaptured later in life documented a mean increase of 520% in the THg concentration from HY to SY age classes, but showed more modest increases from the SY to the third-year age class, and no clear trend in exposure after their third year (i.e., four of six individuals experienced declines in THg as they aged; Barnes et al. 2018).

Concentrations of THg we detected in northern-latitude peregrines were somewhat lower than those documented in recent North American studies, but indicated peregrines across the continent have been exposed to Hg regardless of region or subspecies. We detected overall mean THg concentrations of 2.72  $\mu\text{g/g}$  and 10.29  $\mu\text{g/g}$  in p4 feathers in HY and

AHY peregrines, respectively. Our samples derive primarily from the northern latitudes of North America encompassed by the eastern and western portions of the *F. p. tundrius* subspecies' range, and from the northern distribution of *F. p. anatum* (Yates et al. 1988, Johnson et al. 2010, White et al. 2013; Fig. 1). By comparison, a recent assessment at breeding territories of a nonmigratory population of *F. p. anatum* in southern Nevada in the southwestern USA documented mean THg concentrations of 3.76  $\mu\text{g/g}$  in HY peregrines and 12.19  $\mu\text{g/g}$  in AHY peregrines (Barnes and Gerstenberger 2015). An assessment of migrating and wintering *F. p. pealei* peregrines along coastal Washington detected mean THg concentrations of 6.05  $\mu\text{g/g}$  and 23.11  $\mu\text{g/g}$  in feathers of HY and AHY individuals, respectively ( $n = 151$ ; Barnes et al. 2018).

Diet was unknown, so we could not determine primary Hg exposure pathways; however, earlier research in the northern latitudes of North America indicated the bulk of peregrine prey was composed of migrant land birds or aquatic birds (Hunter et al. 1988, Rosenfield et al. 1995, Dawson et al. 2011). The long-distance migration pattern characteristic of the northern-latitude peregrines further complicates an analysis of their Hg exposure pathways. Studies assessing northern-latitude peregrines on migration (Henny et al. 1982) and on their northern breeding grounds (Springer et al. 1984) found the bulk of exposure of these birds to harmful pesticides by the late 1970s was encountered on their southern wintering grounds. It is currently unknown how Hg exposure on our peregrines' wintering grounds (e.g., Central and South America, Caribbean Sea, southern USA; Fuller et al. 1998, White et al. 2013) compares with that on their northern breeding grounds. However, research on Hispaniola has shown elevated Hg concentrations in blood of resident and migratory passerine species in terrestrial forests on the Caribbean island compared to individuals sampled in northeastern USA (Townsend et al. 2013). Furthermore, the current increasing Hg emission trends in northern South America (UNEP 2013, Evers et al. 2016) may have implications for Hg exposure in North American migratory peregrines and their Neotropical migrant prey while they are on their overwintering grounds.

A meta-analysis of Hg exposure in various taxa of Arctic biota over the past 150 yr showed clear increases since 1900 (average of 1–4% per year; AMAP 2011). However, Hg concentrations in Ivory Gulls (Bond et al. 2015) continue to increase, while

concentrations in some terrestrial animals have stabilized since the 1970s (AMAP 2011). When compared to the 150-yr time series of *F. p. tundrius* in West Greenland (Dietz et al. 2006b), our mean THg results (HY = 2.72  $\mu\text{g/g}$ ; AHY = 10.29  $\mu\text{g/g}$ ) indicate Hg concentrations in northern-latitude peregrines have likely increased in recent decades, and certainly since the early 1900s. Dietz et al. (2006b) detected mean Hg concentrations of 0.66  $\mu\text{g/g}$  and 6.11  $\mu\text{g/g}$  in fifth primaries (p5) from HY and AHY individuals, respectively, from their most recent decade of sampling (1994–2004), but inferences were limited by small sample size. The bulk of their samples came from 1860–1930, during which time they detected a 1.1% increase per year of Hg in juvenile peregrines. Parrish et al. (1983) detected mean Hg concentrations of 0.8  $\mu\text{g/g}$  in West Greenland, 3.1  $\mu\text{g/g}$  along the Yukon River, and 1.5  $\mu\text{g/g}$  along the Colville River in fifth secondary (s5) feathers from nestling peregrines in 1979, which is similar to our HY results, but this study also suffered from low sample sizes. There is an inconsistency in feathers sampled in the above studies (i.e., p4, p5, and s5); however, these are all some of the first feathers to be replaced each molt cycle on the breeding grounds (Pyle 2008) and thus they should accumulate comparable Hg concentrations.

Avian toxic effects levels are species-specific (Wolfe et al. 1998) and concentrations that may negatively affect peregrine health or breeding success are currently unknown. However, it appears that the increase of mean THg in feathers of adults from 4.45  $\mu\text{g/g}$  in 1915–1924 in West Greenland (Dietz et al. 2006b) to our current mean of 10.29  $\mu\text{g/g}$  across the northern-latitude breeding area is not currently suppressing the population. A recent synthesis of banding data from 1970–2010 in northern-latitude peregrines indicates a robust breeding population of more than 15,000 breeding pairs (Franke 2016), which is far greater than the pre-DDT era population estimate of 8660–9000 pairs (White et al. 2002). For comparison, the local breeding population of *F. p. anatum* along the Colorado River system in southern Nevada had a mean THg concentration in feathers of 17.24  $\mu\text{g/g}$  (Barnes and Gerstenberger 2015) in a seemingly healthy breeding population experiencing 72% breeding success and 1.8 successful young per breeding attempt (Barnes et al. 2015). Spry and Wiener (1991) suggested feather Hg concentrations of 15  $\mu\text{g/g}$  were associated with decreased repro-

duction in some predatory birds, so further research is needed to account for potential effects of Hg contamination on peregrine mortality and breeding success rates.

In summary, our assessment of axillary and p4 feathers from peregrines sampled during autumn migrations at SPI and AI is a broadscale assessment of Hg exposure in northern-latitude peregrines across their North American breeding grounds, and indicates widespread THg concentrations generally elevated above prior levels in western Greenland (Dietz et al. 2006b). Our results from AHY feathers are primarily indicative of THg body burdens during the breeding season, likely influenced by uptake throughout the annual cycle. Axillary feather concentrations are highly correlated with concentrations of THg in p4 feathers in juvenal plumage, and we have used them to provide a minimum relative index of Hg exposure throughout peregrines' northern-latitude breeding grounds. However, they are not replaced in a predictable pattern, and so do not provide standardized results for direct comparison in older individuals.

We recommend testing the p4 in peregrines, the first flight feather to be replaced each year in AHY individuals, which enables spatial and temporal analyses of Hg exposure associated with breeding areas and annual exposure in migratory individuals. This is particularly so for peregrines, which exhibit strong breeding-site fidelity, and undergo a predictable and complete annual molt cycle (Pyle 2008). We also recommend trend analyses and comparisons between feathers and blood samples collected during migration to help ascertain Hg exposure on migration relative to the remainder of the annual cycle. Toxic effect levels of Hg are unknown for peregrines, so future research would benefit from an effort to study Hg exposure in relation to adult turnover at territories, breeding success, productivity, and survival rates of peregrines breeding in northern latitudes.

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