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Factors influencing mercury exposure in Arctic-breeding shorebirds

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Abstract

Mercury (Hg) pollution remains a concern to Arctic ecosystems, due to long-range transport from southern industrial regions and melting permafrost and glaciers. The objective of this study was to identify intrinsic, extrinsic, and temporal factors influencing Hg concentrations in Arctic-breeding shorebirds and highlight regions and species at greatest risk of Hg exposure. We analyzed 1094 blood and 1384 feather samples from 12 shorebird species breeding at nine sites across the North American Arctic during 2012 and 2013. Blood Hg concentrations, which reflect Hg exposure in the local area in individual shorebirds: 1) ranged from 0.01–3.52 μg/g ww, with an overall mean of 0.30 ± 0.27 μg/g ww; 2) were influenced by species and study site, but not sampling year, with birds sampled near Utqiaġvik, AK, having the highest concentrations; and 3) were influenced by foraging habitat at some sites. Feather Hg concentrations, which reflected Hg exposure from the wintering grounds: 1) ranged from 0.07–12.14 μg/g fw in individuals, with an overall mean of 1.14 ± 1.18 μg/g fw; and 2) were influenced by species and year. Most Arctic-breeding shorebirds had blood and feather Hg concentrations at levels where no adverse effects of exposure were predicted, though some individuals sampled near Utqiaġvik had Hg levels that would be considered of concern. Overall, these data increase our understanding of how Hg is distributed in the various shorebird breeding areas of the Arctic, what factors predispose Arctic-breeding shorebirds to Hg exposure, and lay the foundation for future monitoring efforts.

Keywords Wildlife · Bioindicators · Arctic · Shorebird · Mercury exposure · Avian

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Introduction

Mercury (Hg) is a pervasive environmental contaminant known to pose health risks to wildlife at elevated levels (Eagles-Smith et al. 2018; UNEP 2019, Grunst et al. 2023). Anthropogenic Hg emissions continue to rise globally and are highly mobile in the atmosphere, traveling great distances and deposited in remote regions, such as the Arctic (AMAP 2021, UNEP 2019, AMAP/UNEP 2015). Once deposited across the landscape, Hg can be converted into methylmercury (MeHg), a more toxic and biologically available form of Hg that biomagnifies through the food web (Eagles-Smith et al. 2018). Chronic exposure to MeHg can impair the physiology and behavior, and dramatically affect the reproductive success, of birds and other wildlife (Scheuhammer et al. 2011, Ackerman et al. 2016a, Chastel et al. 2022).

Birds have emerged as effective bioindicators of Hg exposure, providing reference levels for Hg risk in various ecosystems, including marine habitats, coastal, estuarine, freshwater wetlands, and upland forests (Goodale et al. 2008, Warner et al. 2010, Jackson et al. 2015). Hg assimilates and accumulates rapidly in the blood and feathers of birds (Bearhop et al. 2000), and these tissues are non-lethally sampled with minimal (blood, Lanctot 1994) or presumed low risk (feathers, McDonald and Griffith 2011) to individual birds. The majority of total Hg (THg) within bird blood and feathers is in the form of MeHg (Rimmer et al. 2005, Edmonds et al. 2010). Hg concentrations in avian blood are often suggested to reflect recent exposure, with concentrations increasing rapidly over time for birds fed a diet dosed with MeHg (Bearhop et al. 2000, Fournier et al. 2002, Bennett et al. 2009, Bottini et al. 2021). During feather molt, considered one of the main routes of Hg elimination in addition to depuration during egg laying (Furness et al. 1986, Lewis and Furness 1993), MeHg is transferred from blood to growing feathers (Bottini et al. 2021). As such, MeHg concentrations in feathers are thought to reflect blood MeHg concentrations at the time feathers are being grown (Bearhop et al. 2000, Renedo et al. 2018). In addition, MeHg can be remobilized from other body tissues and deposited in feathers (Honda et al. 1986, Braune 1987, Agusa et al. 2005). Accordingly, feather Hg concentrations are often suggested to reflect exposure from the local environment during feather growth as well as overall body burdens (Wolfe et al. 1998). These differences in the process by which Hg is assimilated into blood and feathers potentially provide insights into Hg exposure at different times and locations along a bird's migratory cycle.

Many species of North American shorebirds are declining, making them a group of conservation concern (Rosenberg et al. 2019, Smith et al. 2023). This concern is noteworthy in the Arctic where the diversity of shorebird species is relatively high and pressures from climate change are amplified (Smith et al. 2020). The influence of Hg on these Arctic-breeding species is not well-known (Chastel et al. 2022); a few previous studies have reported blood Hg concentrations in some shorebird species breeding in the eastern Canadian Arctic (Hargreaves et al. 2010, 2011) and northern Alaska (Perkins et al. 2016) that exceed values associated with adverse effects in other avian species. Thus, there is a need for more research in this area to expand the number of species and locations studied, while also deepening our understanding of exposure pathways and potential risks. The objective of the current study was to measure Hg concentrations in Arctic-breeding shorebird blood and feathers. This objective follows other investigations of Hg exposure for shorebirds (e.g. Braune and Noble 2009, Pratte et al. 2020, Ma et al. 2022). Here, our aim has the potential to help identify vulnerable species and highlight regions within the terrestrial Arctic or non-breeding (a.k.a 'wintering') grounds with the greatest Hg exposure risk. The inclusion of two tissue types provides a more holistic understanding of exposure, given that blood Hg generally reflects recent Hg exposure from the sampled environment, while feather Hg generally reflects Hg exposure where feathers are grown, in this instance, the wintering grounds.

To better understand the considerable variability in bird blood and feather Hg concentrations that have been observed among and within species (Bond and Diamond 2008, Hargreaves et al. 2011, Perkins et al. 2016), we investigated several intrinsic (e.g., sex, body condition, bill length, molt, foraging strategy), extrinsic (e.g., foraging habitat, foraging location), and temporal (e.g., seasonal trends) factors that might influence Hg concentrations of bird blood and feathers (Eagles-Smith et al. 2018). We predicted that foraging location (i.e., breeding or wintering site) would influence blood and feather Hg concentrations in shorebirds given differences in environmental Hg concentrations across the landscape. We predicted also that blood and feather Hg concentrations would vary among species given differences in bill lengths, foraging strategies, and foraging habitat. More specifically, we predicted that shorebird species foraging more frequently in wetter habitats on the breeding grounds would have higher blood Hg concentrations (Keller et al. 2014, Jackson et al. 2015, Perkins et al. 2016). In addition, we predicted that blood and feather Hg concentrations would differ within shorebird species depending on an individual's sex or body condition. Specifically, we predicted that females would exhibit lower blood Hg concentrations due to MeHg deposition in eggs (Robinson et al. 2012). Finally, we predicted that blood Hg concentrations could vary temporally throughout the nesting season due to carry over effects (Lavoie et al. 2014), Hg mobilization due to tissue catabolism during migration

Fig. 1 Sampling sites, part of the Arctic Shorebird Demographics Network, where blood and feather samples were collected from adult shorebirds in 2012 and 2013 for Hg analysis

(Hargreaves et al. 2011, Seewagen et al. 2016), or, for Dunlin molting during the breeding season, deposition of Hg in newly grown feathers (Bearhop et al. 2000).

Methods

Study species and sites

Researchers at field sites involved in the Arctic Shorebird Demographics Network (ASDN), collected shorebird feathers and blood in 2012 and 2013 at five sites across Alaska—Nome, Cape Krusenstern, Utqiaġvik (formerly Barrow), the Ikpikpuk River, and the Colville River—and three sites across the Canadian Arctic—the Mackenzie River Delta (Northwest Territories), Bylot Island (Nunavut), and East Bay (Qaqsauqtuuq, Nunavut) (Fig. 1, Lanctot et al. 2016). In 2013, shorebirds were sampled at an additional study site in Canada—Igloolik (Nunavut). Species composition and abundance varied by site, but 12 shorebird species were sampled across all sites (Table 1). Two subspecies of Dunlin were sampled from Western Alaska: *pacifica*, which winter along the Pacific Coast of North America, and *arcticola*, which winter along the East Asian-Australasian Flyway (Gill et al. 2013), where Hg exposure may be greater. Samples were collected from adult shorebirds (i.e., birds that hatched the prior summer or earlier) captured at nests with bownets (Bub 1995). Nests were found using systematic area searches and behavioral cues of adults, as well as by flushing adults off nests using ropes dragged over suitable tundra areas. A small number of adults were captured during pre-laying or while brooding using mist nets and walk-in traps. Both individuals of mated pairs were sampled when possible. For each bird sampled, the location and date of capture, sex, flight feather molt pattern, and morphometric measurements were recorded.

Sample collection

Blood and feather samples were collected using standard protocols (Evers 2008). Approximately 50–100 µl of blood was collected in heparinized capillary tubes after puncture of the brachial vein using a small gauge needle. No more than 1% of the birds' body weight in blood was collected during sampling (Fair et al. 2010). During field collection, whole blood samples were stored in a cooler with ice packs. At Cape Krusenstern, Utqiaġvik, Colville River, Mackenzie Delta, and Bylot Island, each capillary tube was sealed firmly on both ends with Critocaps (Leica Microsystems, Inc., Concord, Ontario, Canada), placed in a labeled 6 ml plastic vacutainer to prevent breakage, and stored frozen until analysis. At the remaining sites (Nome, Ikpikpuk River, East Bay, and Igloolik), blood from each capillary tube was transferred to a labeled cryovial tube and stored in a dry nitrogen-filled cryoshipper that kept samples below −40 °C. Feathers grown on the wintering grounds were also collected, including a single 10th secondary feather for all species, except for Dunlin where 5 black breast feathers were collected. In contrast to all other species that molt their flight feathers on the non-breeding grounds (Birds of the World 2022), Dunlin molt their flight feathers during the breeding season (Holmes 1966). In general, Dunlin molt their body feathers from late February through early May (Holmes 1966), and thus, breast feather samples were

Table 1 Overview of breeding season foraging habitat, guild, and main food taken for Arctic shorebird species

Species	Foraging Habitat (Moisture Classification)	Foraging Habitat Description	Guild	Main Foods Taken
Black-bellied Plover (*Pluvialis squatarola*) (Poole et al. 2020)	Upland	Open tundra	Invertivore	Insects: Diptera; Coleoptera; Lepidoptera, Ephemeroptera; Trichoptera Some plant matter
American Golden-Plover (*Pluvialis dominica*) (Johnson et al. 2021)	Upland	Various types of tundra	Invertivore; primarily terrestrial	Insects: Various adult and larval insects Berries
Ruddy Turnstone (*Arenaria interpres*) (Nettleship 2020)	Intermediate	Dry to mesic tundra; near to ponds and streams; wet habitats after hatch	Invertivore	Insects: Diptera, primarily Chironomidae Some plant matter
Black Turnstone (*Arenaria melanocephala*) (Handel and Gill 2020)	Intermediate	Wet sedge meadows; shrub mat and alpine tundra	Invertivore; aquatic and terrestrial	Insects: Diptera; Coleoptera Some seeds
Dunlin (*Calidris alpina*) (Warnock and Gill 2020)	Intermediate	Rims and troughs of polygons; hummocks; wet marsh; edges of lakes and ponds	Invertivore; freshwater, marine, and terrestrial	Insects: Diptera, primarily Chironomidae and Tipulidae larvae
Pectoral Sandpiper (*Calidris melanotos*) (Farmer et al. 2020)	Intermediate	Flat, grass-sedge meadows; edges of tundra polygons (wetter than Dunlin)	Invertivore; freshwater, marine, and terrestrial	Insects: Diptera, primarily Chironomidae and Tipulidae
Semipalmated Sandpiper (*Calidris pusilla*) (Hicklin and Gratto-Trevor 2020)	Intermediate	Edges of lakes and ponds	Invertivore; benthic and terrestrial	Insects: Diptera, primarily Chironomidae and Tipulidae Arachnids Some seeds
Western Sandpiper (*Calidris mauri*) (Franks et al. 2020)	Intermediate	Wet mud and shallow water Tundra; littoral zone of ponds	Invertivore; benthic and terrestrial	Insects: Diptera, primarily larval and pupal Muscidae, Chironomidae, and Tipulidae; Coleoptera Arachnids; Grazer on biofilms (diatoms)
Long-billed Dowitcher (*Limnodromus scolopaceus*) (Takekawa and Warnock 2020)	Wet to aquatic	Shallow water of interior wetlands and wet meadows	Invertivore; benthic, aquatic, and terrestrial	Insects: Diptera, primarily Chironomidae larvae; Some plant matter
Red-necked Phalarope (*Phalaropus lobatus*) (Rubega et al. 2020)	Wet to aquatic	Aquatic, small pools and ponds	Invertivore; aquatic	Insects: Diptera, primarily adult and larval Chironomidae, and Tipulidae Small invertebrates; Copepods
Red Phalarope (*Phalaropus fulicarius*) (Tracy et al. 2020)	Wet to aquatic	Shallow water at pond edges	Invertivore; aquatic and terrestrial	Insects: Diptera, primarily adult and larval Chironomidae, and Tipulidae

Information adapted from The Birds of the World (2022) species account for each species, with additional input on moisture classification of predominate foraging habitat (foraging habitat moisture classification; R.B. Lanctot, *personal observation*) assumed to reflect non-breeding ground conditions. Most feather samples were collected by removing the entire feather; however, a small number of feathers were cut at the superior umbilicus. All feather samples were placed in a clean paper envelope and stored at ambient temperature until analysis. Blood samples were shipped to Biodiversity Research Institute's (BRI) Mercury Laboratory on ice in coolers and stored frozen (−20 °C) prior to analysis.

Mercury analysis

Blood and feathers were analyzed for total Hg (THg) concentrations with a direct Hg analyzer (DMA-80, Milestone Inc., Shelton, Connecticut, USA) following United States Environmental Protection Agency Method 7473 (U.S. EPA 2007). Quality control samples were analyzed before and after every set of 30 samples and included one sample each of two Standard Reference Materials (DORM3 fish protein and DOLT-4 fish liver; National Research Council of Canada), two method blanks, and one sample blank. A duplicate shorebird blood sample was analyzed after every set of 20 samples ($n = 37$). Feathers were not washed before analysis, however, external contamination should be minimal compared to internal Hg sources (Goede and DeBruin 1986, Borghesi et al. 2016). The entire secondary feather was analyzed for THg, thus, there was no remaining feather tissue for duplicate feather sample analysis for all shorebird species except Dunlin. For this species, we used the extra breast feathers to repeat analyses on 8 individuals. Duplicate samples were averaged for all statistical analyses. Total Hg concentrations for all sampled shorebirds are reported in micrograms per gram (μg/g) wet weight (ww) for blood, and μg/g fresh weight (fw) for feathers.

For blood and feather Hg analyses, Standard Reference Material sample concentrations were within ± 10% of the expected Hg concentrations. Analytical precision for shorebird blood samples analyzed in duplicate, calculated as mean relative percent difference (RPD), was 1.9 ± 1.6% ($n = 37$). Analytical precision for Dunlin feathers analyzed in duplicate, calculated as meanRPD, was 15.1 ± 11.7% ($n = 8$). All quality control analyses were acceptable according to U.S. Environmental Protection Agency Method 7473 guidelines (U.S. EPA 2007).

Assigning foraging habitat, sex, and molt score

We grouped species by the moisture content of their predominant foraging habitat during the breeding season, as indicated in Birds of the World (2022) species accounts and by field observations (R.B. Lanctot *personal observation*; Table 1). Foraging habitat moisture classification groups consisted of (1) upland, which included Black-bellied Plover (*Pluvialis squatarola*) and American Golden-Plover (*Pluvalis dominica*), (2) intermediate, which included Ruddy Turnstone (*Arenaria interpres*), Black Turnstone (*Arenaria melanocephala*), Dunlin, Pectoral Sandpiper (*Calidris melanotos*), Semipalmated Sandpiper (*Calidris pusilla*), and Western Sandpiper (*Calidris mauri*), and (3) wet to aquatic, which included Long-billed Dowitcher (*Limnodromus scolopaceus*), Red-necked Phalarope (*Phalaropus lobatus*), and Red Phalarope (*Phalaropus fulicarius*, Table 1).

The sex of each shorebird was determined where possible using either (1) breeding characteristics, such as egg laying, brood patch, cloacal protuberance, and known sex of mate, (2) plumage or morphometric measurements, as outlined in Birds of the World (2022) species accounts and other literature (Sandercock 1998), or (3) discriminant function equations (Gates et al. 2013) or molecular analysis. Due to species-specific nest attendance behavior, samples were only obtained from male Red-necked Phalaropes and male Red Phalaropes, and female Pectoral Sandpipers. For all other species, both sexes were sampled. Due to plumage similarities and overlap in morphometric measurements between sexes, the sex of individuals could not be determined in 196 birds, comprising 13.3% of the samples. For the 5 species for which we had 15 or more samples/sex, we compared sex-specific Hg concentrations and calculated mean bill lengths by sex. Females have longer bills than males, but the magnitudes differ among species. This comparison allowed us to investigate whether sex differences in Hg concentrations correlated with dimorphism of bill lengths, which would likely reflect the depth of foraging substrate (e.g. van Gils et al. 2016).

As Dunlin were the only species sampled that may molt their flight feathers during the breeding season (Holmes 1966), we also determined molt scores for each flight feather based on the growth stage of the feather. Scores for individual feathers range from 0 to 5, with a score of 0 indicating the presence of an old feather (i.e., no molt occurring), and a score of 5 indicating a completely grown, new feather (Ginn and Melville 1983). The molt score of each primary, secondary, and tertiary feather was determined for Dunlin sampled at Utqiaġvik and Colville River. We summed the molt scores for all flight feathers to determine an overall individual flight feather molt score, where a higher score indicated a greater amount of flight feather molt had occurred at the time of sampling.

Statistical analyses

We report the mean, standard deviation, and range of Hg concentrations for blood and feathers from shorebird species sampled during each year at each location. We determined that a lognormal distribution best fit the blood and feather Hg data and fit generalized linear mixed-effects

Table 2 Comparisons of blood total Hg concentrations (arithmetic mean, standard deviation (SD), range, and sample size, *n*) for all Arctic shorebird species, sampled at each study site during 2012 and 2013, with the percent of samples in each Hg risk category (years combined where applicable) adapted from toxicity benchmarks proposed by Ackerman et al. (2016a) and Jackson et al. (2011)

						Hg Exposure Risk Categories			
						Background	Low	Low to Moderate	Moderate
Study Site	Species	Year	*n*	Blood Hg (SD)	Blood Hg Range	<0.20	0.20–0.70	0.70–1.0	1.0
Nome	Dunlin	2012	1	0.33	·	0%	100%	0%	0%
	Pectoral Sandpiper	2012	5	0.23 (0.10)	0.16–0.41	40%	60%	0%	0%
	Semipalmated Sandpiper	2012	21	0.17 (0.10)	0.05–0.44	73%	27%	0%	0%
		2013	16	0.16 (0.09)	0.05–0.33				
	Western Sandpiper	2012	15	0.26 (0.09)	0.16–0.48	57%	43%	0%	0%
		2013	13	0.11 (0.07)	0.03–0.30				
	Red-necked Phalarope	2012	16	0.11 (0.03)	0.07–0.18	91%	7%	0%	2%
		2013	28	0.19 (0.29)	0.04–1.55				
Cape Krusenstern	Black Turnstone	2012	15	0.20 (0.13)	0.11–0.63	63%	38%	0%	0%
		2013	1	0.20	·				
	Dunlin	2012	13	0.15 (0.03)	0.09–0.21	94%	6%	0%	0%
		2013	19	0.13 (0.05)	0.03–0.27				
	Semipalmated Sandpiper	2012	34	0.18 (0.04)	0.11–0.27	76%	24%	0%	0%
		2013	8	0.17 (0.04)	0.13–0.24				
	Western Sandpiper	2012	28	0.21 (0.05)	0.11–0.35	57%	43%	0%	0%
		2013	21	0.17 (0.04)	0.09–0.23				
	Red-necked Phalarope	2012	10	0.17 (0.08)	0.07–0.30	70%	30%	0%	0%
Utqiaġvik	American Golden-Plover	2012	20	0.20 (0.07)	0.10–0.40	62%	38%	0%	0%
		2013	14	0.16 (0.07)	0.09–0.35				
	Dunlin	2012	32	0.31 (0.14)	0.15–0.76	13%	83%	3%	0%
		2013	57	0.33 (0.14)	0.09–0.70				
	Pectoral Sandpiper	2012	34	0.58 (0.36)	0.20–2.04	0%	72%	18%	10%
		2013	26	0.54 (0.29)	0.23–1.09				
	Semipalmated Sandpiper	2012	34	0.70 (0.33)	0.18–1.53	1%	59%	24%	15%
		2013	40	0.61 (0.29)	0.25–1.56				
	Western Sandpiper	2012	17	0.43 (0.15)	0.27–0.73	0%	94%	0%	0%
		2013	16	0.40 (0.09)	0.24–0.58				
	Long-billed Dowitcher	2012	23	0.68 (0.17)	0.39–1.01	0%	61%	22%	17%
		2013	13	0.87 (0.33)	0.49–1.49				
	Red-necked Phalarope	2012	10	0.48 (0.35)	0.26–1.43	0%	93%	0%	7%
		2013	4	0.34 (0.08)	0.29–0.46				
	Red Phalarope	2012	36	0.42 (0.23)	0.12–1.12	10%	74%	12%	4%
		2013	32	0.54 (0.31)	0.20–1.60				
Ikpikpuk River	Dunlin	2012	21	0.06 (0.03)	0.01–0.12	100%	0%	0%	0%
		2013	11	0.11 (0.03)	0.07–0.19				
	Semipalmated Sandpiper	2012	46	0.08 (0.04)	0.03–0.20	90%	10%	0%	0%
		2013	31	0.16 (0.06)	0.10–0.32				
	Red-necked Phalarope	2012	15	0.07 (0.03)	0.03–0.17	96%	4%	0%	0%
		2013	12	0.15 (0.11)	0.08–0.49				
	Red Phalarope	2012	19	0.07 (0.03)	0.04–0.16	100%	0%	0%	0%
		2013	13	0.14 (0.03)	0.09–0.19				

Table 2 (continued)

						Hg Exposure Risk Categories			
						Background	Low	Low to Moderate	Moderate
Colville River	Ruddy Turnstone	2013	12	0.61 (0.95)	0.13–3.52	33%	50%	0%	17%
	Dunlin	2012	12	0.17 (0.04)	0.13–0.24	80%	20%	0%	0%
		2013	13	0.18 (0.05)	0.13–0.31				
	Semipalmated Sandpiper	2012	14	0.25 (0.08)	0.17–0.41	38%	63%	0%	0%
		2013	2	0.18 (0.01)	0.17–0.19				
	Red-necked Phalarope	2013	8	0.26 (0.09)	0.13–0.39	38%	63%	0%	0%
	Red Phalarope	2012	13	0.26 (0.09)	0.11–0.38	23%	77%	0%	0%
Mackenzie River Delta	Pectoral Sandpiper	2013	7	0.36 (0.21)	0.15–0.66	29%	71%	0%	0%
	Semipalmated Sandpiper	2012	16	0.19 (0.04)	0.12–0.27	64%	36%	0%	0%
		2013	20	0.19 (0.05)	0.11–0.28				
	Red-necked Phalarope	2012	15	0.24 (0.08)	0.13–0.45	13%	87%	0%	0%
		2013	23	0.37 (0.10)	0.21–0.64				
Bylot Island	American Golden-Plover	2012	23	0.13 (0.05)	0.06–0.30	96%	4%	0%	0%
		2013	24	0.11 (0.03)	0.08–0.22				
	Pectoral Sandpiper	2013	1	0.32	·	0%	100%	0%	0%
Igloolik	American Golden-Plover	2013	9	0.20 (0.06)	0.10–0.32	56%	44%	0%	0%
	Baird’s Sandpiper	2013	1	0.11	·	100%	0%	0%	0%
	Semipalmated Sandpiper	2013	7	0.43 (0.18)	0.26–0.79	0%	86%	14%	0%
East Bay	Black-bellied Plover	2012	6	0.33 (0.06)	0.25–0.41	0%	100%	0%	0%
		2013	2	0.40 (0.06)	0.36–0.44				
	Ruddy Turnstone	2012	14	0.38 (0.22)	0.16–1.00	13%	81%	0%	6%
		2013	2	0.50 (0.06)	0.46–0.55				
	Red Phalarope	2013	10	0.52 (0.13)	0.28–0.66	0%	100%	0%	0%

See Online Resource 3 for the entire dataset

models (GLMM) and generalized linear models (GLM) with a lognormal distribution on non-transformed Hg concentrations. For all models, we started with the full model (see Online Resource 1), models were compared, and the best model was selected using Akaike’s information criteria (AIC). To estimate the strength of the relationship for each model, we used explained deviance, a log-likelihood proxy for explained variance, calculated as: (Null deviance-Residual deviance)/Null Deviance)*100 (Zuur et al. 2009). Tukey’s HSD post hoc tests were used as appropriate to discern differences across groups. Separate models were fit for blood and feather Hg concentrations with specific details on these provided in Online Resources 1 and 2. For model results, we present Hg concentrations as least squares means with standard errors. We excluded Baird’s Sandpipers (*Calidris bairdii*) from analyses because only 2 samples were available (1 blood and 1 feather sample). Statistical analyses were performed using R (R Core Team 2017), and significance was determined if $P < 0.05$. For GLMM, we used the glmer function in package lme4 (Bates et al. 2016) and for GLM we used the glm function in the R base package (R version 3.3.3, R Core Team 2017).

Mercury risk categories

Adverse effects of Hg exposure on birds have been well documented (Scheuhammer et al. 2011, Ackerman et al. 2016a, Chastel et al. 2022). However, laboratory studies have shown that Hg sensitivity can vary among bird species (Heinz et al. 2009), and risk benchmarks for most species, including shorebirds, are unknown. A comprehensive summary of Hg exposure benchmarks (translated to blood Hg-equivalent ww units) and risk categories, using wild bird and laboratory studies, was compiled by Ackerman et al. (2016a). We applied these risk categories to blood Hg concentrations found for Arctic-breeding shorebirds. We added an additional risk category based on findings from Jackson et al. (2011), which determined blood Hg concentrations of 0.7 µg/g ww were associated with a 10% reduction in nest success for the Carolina Wren

Table 3 Comparisons of feather total Hg concentrations (arithmetic mean, standard deviation (SD), range, and sample size, *n*) for all Arctic shorebird species, sampled at each study site during 2012 and 2013, with the percent of samples in each Hg risk category (years combined where applicable) adapted from toxicity benchmarks determined for the Carolina Wren (Jackson et al. 2011)

						Hg Exposure Risk Categories		
						Low	Moderate	High
Study Site	Species	Year	*n*	Feather Hg (SD)	Feather Hg Range	<3.0	3.0–9.1	>9.1
Nome	Dunlin	2012	2	0.88 (0.41)	0.59–1.17	100%	0%	0%
	Pectoral Sandpiper	2012	7	1.66 (0.89)	0.66–3.40	86%	14%	0%
	Semipalmated Sandpiper	2012	38	0.93 (0.66)	0.28–3.66	99%	1%	0%
		2013	35	0.97 (0.66)	0.18–2.54			
	Western Sandpiper	2012	38	0.97 (0.58)	0.17–2.44	99%	1%	0%
		2013	30	1.03 (0.92)	0.15–4.68			
	Red-necked Phalarope	2012	44	1.03 (0.64)	0.30–2.42	100%	0%	0%
		2013	33	0.60 (0.43)	0.24–2.17			
Cape Krusenstern	Black Turnstone	2012	14	1.69 (1.39)	0.51–4.69	84%	16%	0%
		2013	5	1.32 (1.66)	0.39–4.27			
	Dunlin	2012	14	1.17 (0.71)	0.47–2.54	94%	3%	3%
		2013	19	1.41 (1.32)	0.49–6.38			
	Semipalmated Sandpiper	2012	34	0.79 (0.60)	0.17–2.70	100%	0%	0%
		2013	27	0.72 (0.50)	0.18–2.10			
	Western Sandpiper	2012	27	1.74 (2.83)	0.13–10.24	86%	12%	2%
		2013	30	1.46 (1.13)	0.12–4.42			
	Red-necked Phalarope	2012	9	0.70 (0.33)	0.34–1.33	100%	0%	0%
Utqiaġvik	American Golden-Plover	2012	20	0.60 (0.25)	0.37–1.41	100%	0%	0%
		2013	16	0.75 (0.38)	0.23–1.71			
	Dunlin	2012	24	1.66 (1.20)	0.10–5.34	82%	18%	0%
		2013	60	1.72 (0.79)	0.57–4.87			
	Pectoral Sandpiper	2012	40	2.31 (1.76)	0.56–6.67	70%	30%	0%
		2013	33	3.21 (1.92)	0.94–7.37			
	Semipalmated Sandpiper	2012	55	0.96 (1.30)	0.24–9.25	99%	1%	1%
		2013	81	0.86 (0.63)	0.07–2.90			
	Western Sandpiper	2012	19	1.64 (1.71)	0.19–6.21	81%	17%	2%
		2013	33	1.93 (2.40)	0.17–10.13			
	Long-billed Dowitcher	2012	22	2.19 (2.59)	0.31–12.14	92%	6%	3%
		2013	14	1.07 (0.77)	0.31–2.42			
	Red-necked Phalarope	2012	12	0.46 (0.11)	0.24–0.71	100%	0%	0%
		2013	5	0.46 (0.21)	0.26–0.80			
	Red Phalarope	2012	51	0.68 (0.67)	0.14–4.00	99%	1%	0%
		2013	40	0.50 (0.15)	0.21–0.84			
Colville River	Ruddy Turnstone	2013	14	2.25 (2.04)	0.84–7.29	71%	29%	0%
	Dunlin	2012	12	1.36 (0.47)	0.80–2.72	83%	17%	0%
		2013	17	1.92 (1.01)	0.86–4.96			
	Semipalmated Sandpiper	2012	14	0.80 (0.49)	0.34–1.83	100%	0%	0%
		2013	2	0.83 (0.16)	0.72–0.94			
	Red-necked Phalarope	2013	8	0.43 (0.25)	0.27–1.02	100%	0%	0%
	Red Phalarope	2012	13	0.51 (0.35)	0.21–1.54	100%	0%	0%

Table 3 (continued)

						Hg Exposure Risk Categories		
						Low	Moderate	High
Ikpikpuk River	Dunlin	2012	21	1.02 (0.46)	0.49–1.87	100%	0%	0%
		2013	11	1.32 (0.29)	0.92–1.81			
	Semipalmated Sandpiper	2012	46	0.71 (0.75)	0.18–5.18	98%	2%	0%
		2013	36	0.85 (0.77)	0.27–4.79			
	Red-necked Phalarope	2012	15	0.50 (0.24)	0.18–1.03	100%	0%	0%
		2013	16	0.61 (0.57)	0.24–2.30			
	Red Phalarope	2012	19	0.53 (0.27)	0.24–1.19	100%	0%	0%
		2013	16	0.54 (0.27)	0.22–1.20			
Mackenzie River Delta	Pectoral Sandpiper	2013	8	2.23 (0.90)	0.88–3.94	88%	13%	0%
	Semipalmated Sandpiper	2012	17	1.04 (0.61)	0.14–2.01	100%	0%	0%
		2013	20	0.61 (0.38)	0.13–1.73			
	Red-necked Phalarope	2013	23	0.68 (0.48)	0.21–1.78	100%	0%	0%
Bylot Island	American Golden-Plover	2012	26	0.92 (0.54)	0.31–2.27	94%	6%	0%
		2013	25	1.27 (1.13)	0.34–4.34			
	Pectoral Sandpiper	2013	1	1.91	·	100%	0%	0%
Igloolik	American Golden-Plover	2013	15	1.41 (1.41)	0.35–5.83	87%	13%	0%
	Baird's Sandpiper	2013	1	2.38	·	100%	0%	0%
	Semipalmated Sandpiper	2013	11	0.59 (0.52)	0.30–2.10	100%	0%	0%
East Bay	Black-bellied Plover	2012	6	1.70 (0.84)	0.29–2.61	100%	0%	0%
		2013	6	0.86 (0.43)	0.31–1.55			
	Ruddy Turnstone	2012	14	1.15 (0.87)	0.27–3.29	95%	5%	0%
		2013	5	0.73 (0.22)	0.49–0.93			
	Red Phalarope	2013	15	0.77 (0.34)	0.37–1.33	100%		0%

See Online Resource 3 for the entire dataset

(*Thryothorus ludovicianus*). Therefore, we used 5 blood Hg risk categories: (1) <0.2 µg/g ww, background exposure levels; (2) 0.2 to <0.7 µg/g ww, low risk; (3) 0.7 to <1.0 µg/g ww, low to moderate risk; (4) >1.0 µg/g ww, moderate risk; and (5) > 3.0 µg/g ww, high risk (after Ackerman et al. 2016a). Only one individual, a Ruddy Turnstone sampled at Colville River, had a blood Hg concentration in the high-risk category (3.52 µg/g). This individual was included in the moderate risk category (>1.0 µg/g), making a total of 4 risk categories. We determined the percentage of individuals with blood Hg concentrations in each risk category for each site.

Risk benchmarks based on feather Hg concentrations are even less well-known. Jackson et al. (2011) also modeled the association of nest success and feather Hg concentrations in the Carolina Wren, using concentrations for both body and tail feathers. They determined that Carolina Wren body and tail feather Hg concentrations of 2.4 and 3.0 µg/g ww were associated with a 10% reduction in nest success, respectively, and concentrations of 6.2 and 9.1 µg/g ww were associated with a 50% reduction. We sampled secondary flight feathers for most species, making comparisons with tail feather concentrations tenuous, however, we use these concentrations as a best estimate for flight feather Hg risk benchmarks. Therefore, we used 3 flight feather Hg risk categories: (1) < 3.0 µg/g fw, low risk; (2) 3.0 to <9.1 µg/g fw, moderate risk; (3) > 9.1 µg/g fw, high risk. We sampled body feathers for Dunlin only and grouped this species into body feather Hg risk categories 1) <2.4 µg/g fw, low risk; 2) 2.4 to <6.2 µg/g fw, moderate risk; 3) >6.2 µg/g fw, high risk. We determined the percentage of individuals with feather Hg concentrations in each risk category for each site.

Results

Overview

For this study, a total of 1472 Arctic-breeding shorebirds were sampled across the North American Arctic from 20 May to 22 July 2012, and from 28 May to 23 July 2013

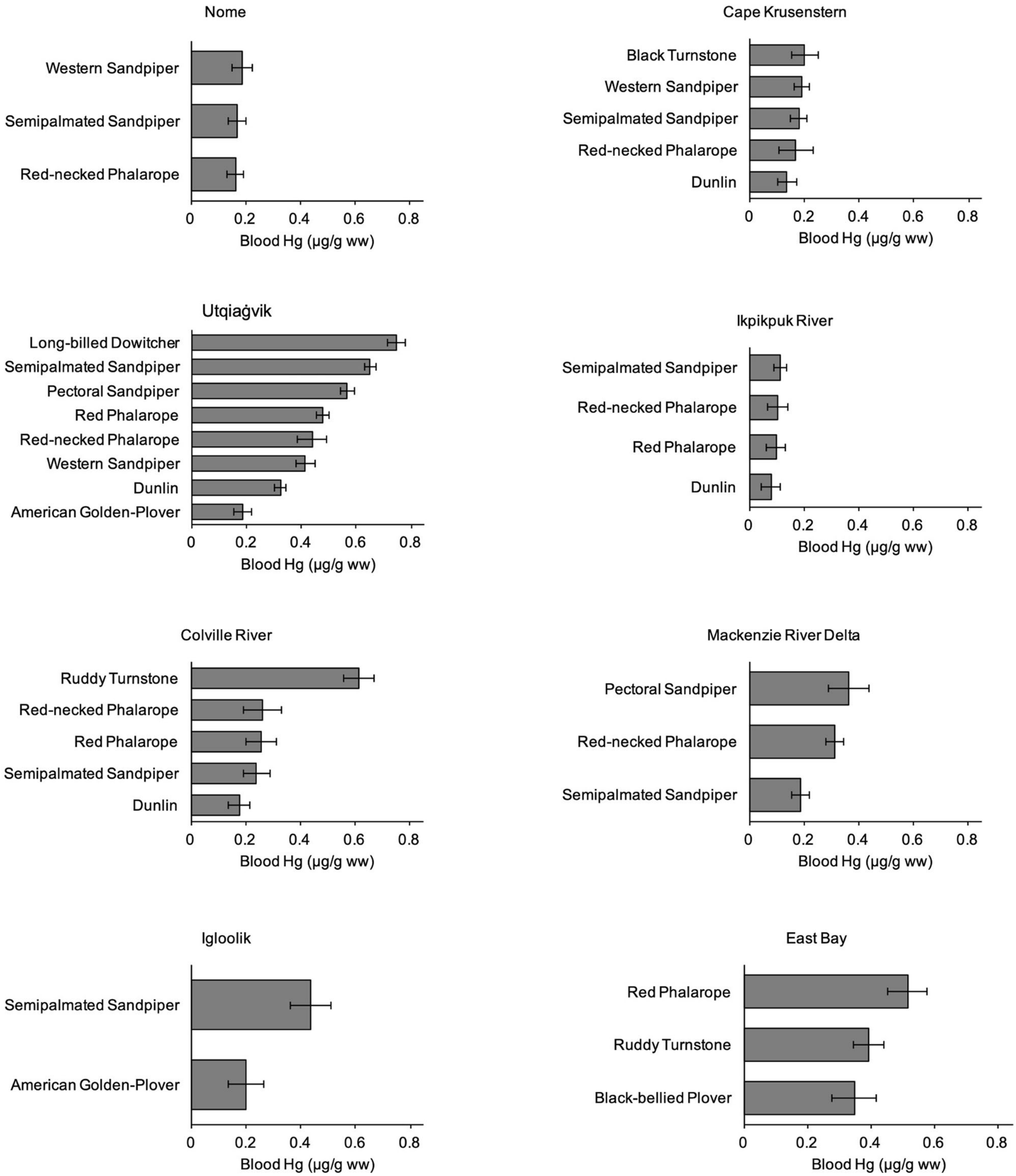

Fig. 2 Comparisons of blood total Hg concentrations (least squares mean and standard error) among shorebird species sampled at Arctic-breeding study sites during 2012 and 2013. Sexes and years combined where applicable. See Fig. 1 for location of study sites, and Table 2 for sample sizes

(Tables 2 and 3; all data in Online Resource 3). We analyzed 1094 blood samples (578 in 2012, and 516 in 2013) and 1384 feather samples (673 in 2012, and 711 in 2013) from 12 shorebird species sampled at 9 study sites (Fig. 1). We sampled the greatest number of individuals ($n = 563$) and the greatest number of species ($n = 8$) at Utqiaġvik in northern Alaska, USA. Relatively lower sample sizes and species numbers were obtained for study sites in eastern

Canada (Bylot Island [$n = 53$, 2 species], Igloolik [$n = 28$, 3 species], and East Bay [$n = 47$, 3 species]).

Blood Hg concentrations

Blood Hg concentrations for all Arctic-breeding shorebirds sampled over the whole area varied considerably, but averaged 0.30 ± 0.27 µg/g ($n = 1093$) and ranged between 0.01 µg/g (Dunlin sampled at Ikpikpuk River) and 3.52 µg/g (Ruddy Turnstone sampled at Colville River, Table 2). Across sites, Utqiaġvik (0.48 ± 0.29 µg/g) had the greatest mean blood Hg concentration in shorebirds, 4.8 times greater than the mean from the site with the lowest concentration, Ikpikpuk River (0.10 ± 0.06 µg/g). Among species across all sites, Long-billed Dowitchers (0.74 ± 0.25 µg/g) had the greatest mean blood Hg concentration, over 4.9 times greater than the species with the lowest concentration, American Golden-Plovers (0.15 ± 0.07 µg/g).

Variability among species, study sites, and year

We examined the influence of the three-way interaction for species, study site, and year on blood Hg concentrations. We found that year was not a significant explanatory variable ($P = 0.53$), and the inclusion of this term did not improve the model. The model including the two-way interaction between species and study site had an explained deviance of 49% and indicated differences were present among species and study sites (Fig. 2). At Utqiaġvik, we saw large differences among species, with Long-billed Dowitchers having an approximately 4.0 times greater least squares mean blood Hg concentration than American Golden-Plover, and 2.3 times greater than Dunlin. At each site where they were sampled, both American Golden-Plover and Dunlin had the lowest least square mean blood Hg concentration. At Igloolik, the least squares mean blood Hg concentration was approximately 2.2 times greater for Semipalmated Sandpipers than for American Golden-Plovers.

Shorebird species sampled at Utqiaġvik had greater Hg exposure than those sampled at other study sites (Fig. 2). For example, Red-necked Phalaropes were sampled at 6 sites and the least squares mean blood Hg concentration at Utqiaġvik was 4.2 times greater than at Ikpikpuk River, and 2.7 times greater than Nome. We saw even greater differences in least squares mean blood Hg concentrations for Semipalmated Sandpipers, with those sampled at Utqiaġvik having 5.7 times and 3.9 times greater concentrations than those sampled at Ikpikpuk River and Nome, respectively. Further comparisons for Semipalmated Sandpipers indicated that the least squares mean blood Hg concentration at Utqiaġvik was ~3.6 and 3.4 times greater than Cape Krusenstern and Mackenzie River Delta, respectively.

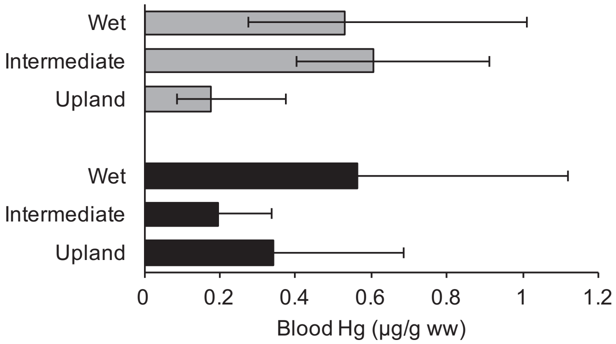

Fig. 3 Comparisons of blood total Hg concentrations (least squares mean and 95% confidence intervals) by preferred foraging habitat moisture classification for Arctic-breeding shorebirds sampled at the Utqiaġvik (light gray) and East Bay (dark gray) study sites. See Fig. 1 for location of study sites, and Table 2 for sample sizes

Influence of foraging habitat and study sites

We found the full model best explained measured blood Hg concentrations, including foraging habitat moisture classification, study site, and the interaction between foraging habitat and study site as fixed factors, and species and year as random factors. A model with the interaction term removed, indicated that foraging habitat was not a significant factor influencing blood Hg concentrations across all study sites (post-hoc comparisons: upland/intermediate, $P = 0.34$; wet/intermediate, $P = 0.98$; wet/upland, $P = 0.37$). However, when the interaction term was included, we observed that blood Hg concentrations were influenced by foraging habitat at Utqiaġvik and East Bay only (Fig. 3). This interaction indicated there is not a clear trend for the influence of foraging habitat on blood Hg concentrations in Arctic-breeding shorebirds. The model results show the least squares mean blood Hg concentrations are greatest for the wet habitat classification at East Bay (0.56 ± 0.02 µg/g), but greatest for the intermediate habitat classification at Utqiaġvik (0.61 ± 0.13 µg/g). The inclusion of the interaction term also indicated that blood Hg concentrations differed among study sites in relation to foraging habitat. We found that Utqiaġvik had the greatest least squares mean blood Hg concentration for species foraging in intermediate habitat, while East Bay had the greatest concentrations for both upland (0.34 ± 0.12 µg/g) and wet foraging species. Bylot Island had the lowest least squares mean blood Hg concentration for the upland habitat (0.11 ± 0.05 µg/g) while Ikpikpuk River, followed by Nome, had the lowest blood Hg concentrations for the intermediate (0.11 ± 0.03 µg/g and 0.19 ± 0.05 µg/g, respectively) and wet habitats (0.11 ± 0.04 µg/g and 0.19 ± 0.07 µg/g, respectively; Table 2).

Influence of capture day and body mass

The model investigating the influence of Julian capture day and body mass on blood Hg concentrations indicated a significant, decreasing relationship for both Julian day ($P = 0.003$) and body mass ($P = 0.03$). These decreases in blood Hg concentrations differed by species and study site. However, the relationship for this model was relatively weak, with an explained deviance of approximately 2%.

Influence of sex

We examined the influence of sex on blood Hg concentrations for American Golden-Plover, Dunlin, Semipalmated Sandpiper, Western Sandpiper, and Long-billed Dowitcher. We found the interaction between sex and species was not significant within the full model. The best model included sex and species as the explanatory variables, with an explained deviance of 28%. Overall, blood Hg concentrations in males were ~11% greater than females, though, within each species, sexes did not differ significantly (Fig. 4a).

Influence of flight feather molt in Dunlin

The overall model investigating the influence of flight feather molt on blood Hg concentrations for Dunlin was significant, however, neither flight feather molt score ($P = 0.61$) nor year ($P = 0.21$) were explanatory variables within the model. The best model explaining blood Hg concentrations in Dunlin sampled at Utqiaġvik and Colville River included only study site and sex, with an explained deviance of 23%. From this model, we determined that the least squares mean blood Hg concentration in Dunlin was ~47% greater at Utqiaġvik (0.32 ± 0.01 µg/g) than at Colville River (0.17 ± 0.02 µg/g, $P < 0.0001$), and ~15% greater in male Dunlin (0.25 ± 0.02 µg/g) than in females (0.21 ± 0.02 µg/g, $P = 0.03$).

Feather Hg concentrations

Feather Hg concentrations (mean: 1.14 ± 1.18 µg/g, $n = 1383$ samples) in Arctic-breeding shorebirds were on average nearly 4× greater than blood and varied greatly across individual shorebirds, with the lowest concentration of 0.07 µg/g detected in a Semipalmated Sandpiper and the highest concentration of 12.14 µg/g in a Long-billed Dowitcher (both sampled at Utqiaġvik; Table 3). As with blood Hg concentrations, mean feather Hg concentrations were greatest at Utqiaġvik (1.35 ± 1.48 µg/g) and lowest at Ikpikpuk River (0.75 ± 0.61 µg/g), with concentrations 1.8 times greater at the Utqiaġvik site. Feather Hg concentrations also differed by species, with the mean feather Hg concentration greatest in Pectoral Sandpipers (2.58 ± 1.76 µg/g), over 4.3 times greater than in Red Phalaropes (0.60 ± 0.44 µg/g) which had the lowest concentrations.

Variability among species, study sites, and year

We examined the influence of the three-way interaction among species, study site, and year on feather Hg concentrations. The model fit was not improved by the inclusion of the study site, and this variable was removed from the model. The model including the two-way interaction between species and year had an explained deviance of 21% and indicated that variability in feather Hg concentrations between 2012 and 2013 differed by species sampled (Fig. 5). In both 2012 and 2013, Pectoral Sandpipers had the greatest least squares mean feather Hg concentration, while Red Phalaropes had the lowest, though the extent of the difference varied by year. Pectoral Sandpipers had approximately 3.6 and 5.3 times greater least squares mean feather Hg concentration than Red Phalaropes in 2012 and 2013, respectively. There was no consistent year effect across species.

Variability between Dunlin subspecies

Because we sampled breast and not secondary feathers in Dunlin, we conducted an independent analysis on the two subspecies sampled in Alaska. The least squares mean feather Hg concentration was greater for *C. a. arcticola* (male: 1.57 ± 0.10 µg/g, female: 1.45 ± 0.11 µg/g) than for *C. a. pacifica* (male: 1.18 ± 0.20 µg/g, female: 1.36 ± 0.22 µg/g). However, neither subspecies ($P = 0.69$), sex ($P = 0.39$), nor the interaction between subspecies and sex ($P = 0.38$) were significant explanatory variables within the model. The best model explaining feather Hg concentrations in Dunlin included year only, though the relationship was weak, with an explained deviance of 4%. The least squares mean feather Hg concentration was ~21% greater in 2013 than in 2012 for Dunlin.

Influence of sex

We examined the influence of sex on feather Hg concentrations in American Golden-Plover, Dunlin, Semipalmated Sandpiper, Western Sandpiper, and Long-billed Dowitcher. The best model included sex, species, and the interaction between sex and species as the explanatory variables, with an explained deviance of 9%. Overall, least squares mean feather Hg concentrations for males ranged from 4% greater than for females in Dunlin to 35% greater than for females in Western Sandpipers (Fig. 4b). The Long-billed Dowitcher was the only species in which females had a least squares mean feather Hg concentration greater (~31%) than males.

The difference in absolute values between sexes for both bill length and feather Hg concentration were largest for Long-billed Dowitcher (Δ8.79 mm, Δ0.68 Hg µg/g; both

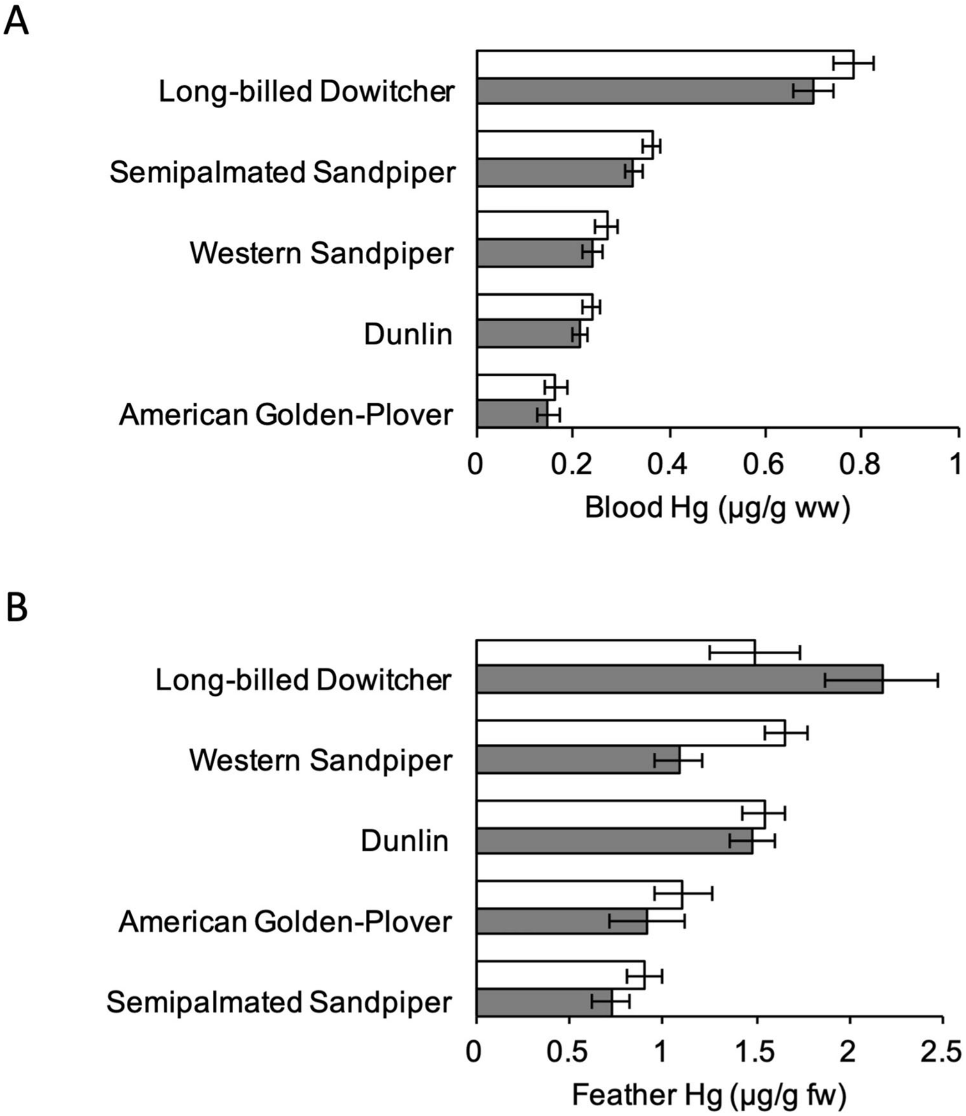

Fig. 4 Comparisons of (**A**) blood and (**B**) feather total Hg concentrations (least squares mean and standard error) between male (white bars) and female (gray bars) shorebirds sampled at Arctic breeding study sites during 2012 and 2013. Years and study sites combined for all species. See Tables 2 and 3 for sample sizes for blood and feathers, respectively

higher in females) and Western Sandpiper (Δ4.32 mm higher in females, Δ0.57 μg/g higher in males; Online Resource 4). The difference in feather Hg concentrations were smaller for the two species with the most similar bill lengths, American Golden-Plover (Δ1.85 mm, Δ0.20 μg/g), and Semipalmated Sandpiper (Δ1.90 mm, Δ0.18 μg/g). But Dunlin, with an intermediate bill length difference, had the smallest difference in Hg feather concentrations (Δ3.21 mm, Δ0.06 μg/g).

Mercury risk categories

Overall, most Arctic-breeding shorebirds had blood Hg concentrations related to the background and low-risk categories for adverse effects of Hg exposure, with ~45% of individuals falling into the background category and 47% falling into the low-risk category (Table 2). We found a low proportion of individuals with blood Hg concentrations within the low to moderate (5% of individuals) and the moderate risk categories (3%). The greatest proportion of individuals in these two categories was sampled at Utqiaġvik (Fig. 6a). The Cape Krusenstern, Ikpikpuk River, Mackenzie River Delta, and Bylot Island sites did not have any individual shorebirds with blood Hg concentrations falling within these higher risk categories. Long-billed Dowitcher had the greatest proportion of individuals in the low to moderate risk and moderate risk categories, with approximately 22 and 17%, respectively (Table 2). Pectoral Sandpiper also had approximately 15 and 8% of individuals within these respective categories. Individual American Golden-Plover, Baird’s Sandpiper, Black-bellied Plover, and Black Turnstone were found to have blood Hg concentrations only within the background and low-risk categories.

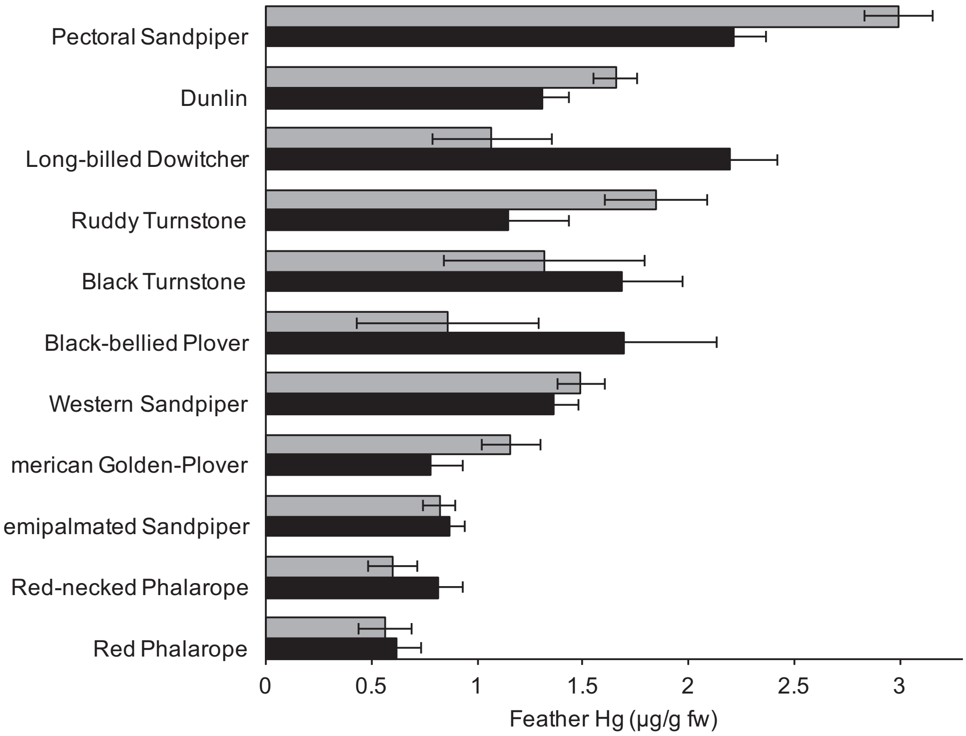

Fig. 5 Comparisons of feather total Hg concentrations (least squares mean and standard error) among shorebird species sampled at Arctic-breeding study sites during 2012 (dark gray bars) and 2013 (light gray bars). Concentrations differed by species and by year ($P < 0.05$). Study sites and sexes combined where applicable. See Table 3 for sample sizes

Feather Hg concentrations also indicated that the majority of Arctic-breeding shorebirds sampled were at low risk for adverse effects from Hg exposure. Approximately 6% of individuals had feather Hg concentrations in the moderate and high-risk categories, with 94% of individuals in the low-risk category (Table 3). Only the Cape Krusenstern and Utqiaġvik study sites had individual shorebirds with feather Hg concentrations in the high-risk category (Fig. 6b). Four species had individuals with feather Hg concentrations in the high-risk category: Dunlin (<1%), Long-billed Dowitcher (~3%), Semipalmated Sandpiper (<1%), and Western Sandpiper (~1%, Table 3). Pectoral Sandpipers did not have any individuals within the high-risk category, but had the greatest proportion of individuals within the moderate-risk category (27%), while individual Baird’s Sandpipers, Black-bellied Plovers, and Red-necked Phalaropes had feather Hg concentrations in the low-risk category only.

Discussion

This study provides Hg concentrations for shorebird blood and feathers, an essential step to estimate mercury exposure (and potential risk) in Arctic species that are of conservation concern. The data can also serve as baselines for evaluating future changes in shorebird exposure to further global Hg emissions and Hg released by permafrost thawing and glacial melting, particularly in light of the Minamata Convention on Hg (UNEP 2013).

Previous large-scale Hg exposure assessments of birds have converted Hg concentrations from various tissues to blood-equivalent units for comparisons, or have used differing sample collection, preparation, and analysis methods (Jackson et al. 2015, 2016, Ackerman et al. 2016a). In this study, we used standardized sample collection methods, analysis methods, and quality control practices for all blood and feather samples, offering an advantage for comparisons among samples. In addition, previous studies have found that foraging guild and ecoregion (i.e., freshwater wetlands, upland forests, salt marsh, etc.) are important factors influencing blood and feather Hg concentrations (Keller et al. 2014, Jackson et al. 2015, Ackerman et al. 2016a). Since the shorebird species sampled in this study forage in Arctic tundra wetlands and occupy the same foraging guild (invertivores, Table 1; Colwell 2010, Birds of the World 2022), we were able to investigate other factors influencing Hg exposure without having to account for ecoregion and foraging guild differences.

Blood Hg concentrations in shorebirds

Overall, shorebirds sampled in this study had low blood Hg concentrations, though we found considerably elevated exposure at some study sites and for certain species. A previous study investigated Hg exposure in breeding shorebirds at Utqiaġvik during 2008 and 2009 using similar procedures (Perkins et al. 2016). In comparison to the current study, mean blood Hg concentrations were similar

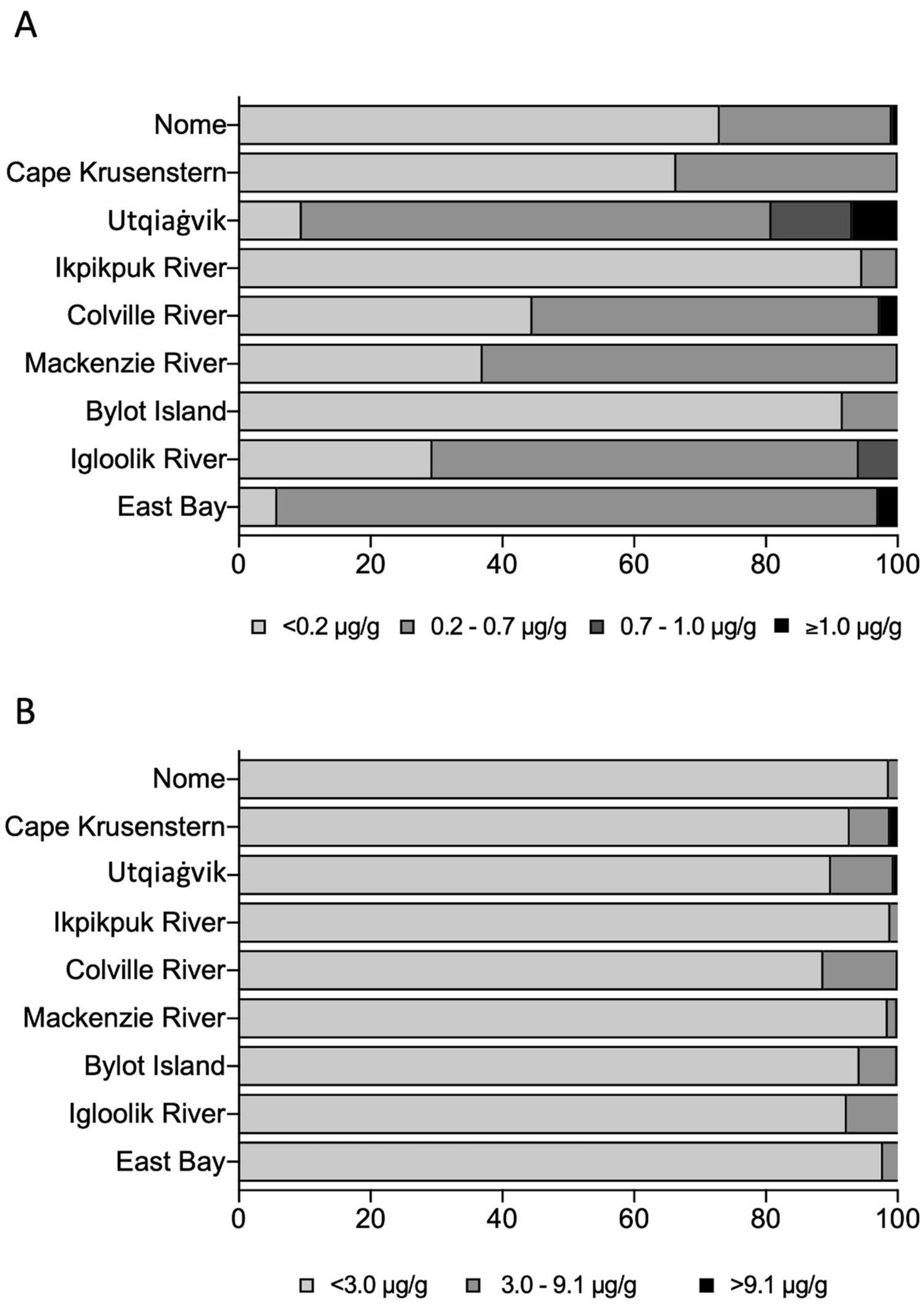

Fig. 6 Comparison of the percentage of individual shorebirds at each study site with (**A**) blood Hg and (**B**) feather Hg concentrations falling within risk categories for adverse effects of Hg exposure. All species and years included. Risk categories for blood Hg were: 1) <0.2 µg/g, background exposure levels; 2) 0.2 to <0.7 µg/g, low risk; 3) 0.7 to <1.0 µg/g, low to moderate risk; and 4) >1.0 µg/g, moderate risk (adapted from Jackson et al. 2011, Ackerman et al. 2016a). Risk categories based on flight feather concentrations 1) <3.0 µg/g, low risk; 2) 3.0 to <9.1 µg/g, moderate risk; 3) >9.1 µg/g, high risk. For Dunlin only, risk categories based on body feather concentrations: 1) <2.4 µg/g, low risk; 2) 2.4 to <6.2 µg/g, moderate risk; 3) >6.2 µg/g, high risk (adapted from Jackson et al. 2011). See Fig. 1 for location of study sites

for American Golden-Plovers (2009: 0.18 ± 0.20 µg/g) and Red Phalaropes, though concentrations were variable among years for this species (2008: 0.66 ± 0.36 µg/g, 2009: 0.43 ± 0.34 µg/g). Pectoral Sandpipers and Semipalmated Sandpipers sampled in the current study had approximately 18% (2009: 0.68 ± 0.66 µg/g) and 31% (2009: 0.95 ± 0.62 µg/g,) lower mean blood Hg concentrations, respectively, than those sampled in the previous study. In contrast, Long-billed Dowitchers and Dunlin had ~46% (2009: 0.53 ± 0.20 µg/g) and 56% (2009: 0.21 ± 0.09 µg/g) greater mean Hg concentrations, respectively, in the current study. Overall, both studies found elevated blood Hg concentrations for shorebirds sampled at Utqiaġvik.

Likewise, blood Hg concentrations were also determined for Arctic-breeding shorebirds at East Bay in 2008 and 2009 using similar procedures (Hargreaves et al. 2010, 2011). In comparison to the current study, these past studies found similar mean blood Hg concentrations in Black-bellied Plovers (means ranged from 0.34–0.43 µg/g) and Red Phalaropes (0.51 ± 0.07 µg/g). However, these previous studies found higher mean blood Hg concentrations in Ruddy Turnstones (means ranged from 0.52–0.68 µg/g), with an ~36% greater concentration seen in 2009 compared to 2012. Comparisons with these previous studies indicate similar Hg exposure for shorebirds sampled at these two sites, however, concentrations for some species varied among years.

These previous studies at Utqiaġvik and East Bay also found similar differences among species of Arctic-breeding shorebirds sampled within the same study site (Hargreaves et al. 2010, 2011, Perkins et al. 2016). While such differences were similarly observed in the current study, we also saw some common trends in blood Hg concentrations among species, regardless of sampling location. For example, at each site where American Golden-Plover and Dunlin were sampled, they had low mean blood Hg concentrations compared to other species. Similarly, species comparisons indicated that Pectoral Sandpipers and Semipalmated Sandpipers had greater mean blood Hg concentrations. This study further emphasizes the importance of species when using birds as bioindicators of Hg risk in a system, even within the same foraging guild and ecoregion. These results suggest that sampling a single species, such as American Golden-Plover, may not identify potential hotspots for Hg exposure, such as Utqiaġvik, since this species had low concentrations across all sites. In contrast, the inclusion of Semipalmated Sandpipers in this study highlighted the potential for high Hg exposure at Utqiaġvik.

We also found significant variability of blood Hg concentrations among sampling sites, indicating that breeding site exposure is an important factor influencing blood Hg concentrations. However, differences in blood Hg concentrations among species, combined with differences in species sampled at each site, make determining Hg exposure risk across study sites difficult. For instance, American Golden-Plover was the only species sampled at the Bylot Island study site, and since this species had low Hg concentrations across all study sites, we are uncertain of the general Hg exposure risk at Bylot Island for other bird species. Future sampling of additional species at this site might provide a better assessment of the exposure risk at this site. Low sample sizes and the number of species sampled at both the Igloolik and East Bay sites also limit understanding of Hg exposure risk at these sites. Overall, we found blood Hg concentrations were highest in species sampled at Utqiaġvik, while Ikpikpuk, located just 100 km southeast of Utqiaġvik, had the lowest blood Hg concentrations. Further research is needed to determine why species breeding at Utqiaġvik had higher Hg exposure, particularly when compared to the nearby Ikpikpuk.

Recent studies focused on Hg exposure in invertivore songbirds suggest that within the terrestrial environment, wetter foraging habitats are associated with greater blood and feather Hg concentrations (Keller et al. 2014, Jackson et al. 2015). Therefore, we expected shorebird species foraging more frequently in wetter habitats to have higher blood Hg concentrations. Indeed, the previous study at Utqiaġvik found higher blood Hg concentrations in species foraging predominately in wetter habitats (Perkins et al. 2016). However, in the current study, we found moisture content of the preferred foraging habitat influenced blood Hg concentrations at Utqiaġvik and East Bay, but there was no consistent pattern between sites. While upland foraging species at Utqiaġvik had the lowest blood Hg concentrations, intermediate foraging species had the lowest blood Hg concentrations at East Bay. These results suggest that foraging habitat differences other than moisture content may have a greater influence on blood Hg concentrations in shorebirds. However, differences in the species sampled at Utqiaġvik ($n = 8$ species) and East Bay ($n = 3$ species), and the resulting difference of species within the foraging habitat groups, may also have played a role.

While shorebirds sampled in this study generally forage on similar major prey items (Table 1), differences in foraging strategy among species may influence blood Hg concentrations. Comparatively little is known about the distribution of MeHg concentrations among invertebrates in the Arctic, though MeHg concentrations in invertebrates have been shown to differ by developmental stage, foraging behavior, trophic level, and feeding depth (Barst et al. 2022). Therefore, differences among shorebird species in foraging and specific prey selection may play a role in blood Hg concentrations. For instance, Long-billed Dowitchers, which had the greatest blood Hg concentrations, forage by probing with their long bill (Takekawa and Warnock 2000) and may forage on invertebrates located deeper in sediments. Sizmur et al. (2013) found polychaete worms in the Bay of Fundy located deeper in sediments had greater MeHg concentrations. In contrast, American Golden-Plovers take prey at the substrate surface (Johnson and Connors 2010). It is currently unknown how these differences in foraging may influence blood Hg concentrations in Arctic-breeding shorebirds. Further research is needed to better understand MeHg distribution within Arctic invertebrate food webs and how shorebird species differ in foraging strategies.

We found a significant, but weak relationship that indicated blood Hg concentrations for Arctic-breeding shorebirds decreased over the sampling period. This result may suggest shorebirds showed some carry over of Hg exposure from wintering grounds or stop-over sites to the breeding season and have lower Hg exposure on the Arctic breeding grounds. Previous studies show carry over from the winter season to the breeding season for two migratory marine bird species, Double-crested Cormorant (*Phalacrocorax auritus*) and Caspian Tern (*Hydroprogne caspia*), though the amount of carry over differed between species (Lavoie et al. 2014). While this may also occur for Arctic-breeding shorebirds, this explanation is contradicted by the much stronger relationship found between blood Hg concentrations and breeding site. While migratory connectivity is not well known for most Arctic-breeding shorebirds, a study using geolocators on Semipalmated Sandpipers compared migratory pathways and wintering locations for individuals from 6 breeding sites used in the current study (Brown et al. 2017). While sample sizes of tracked individuals were low ($n = 3$) for the Ikpikpuk River site, results indicated that Semipalmated Sandpipers breeding at Utqiaġvik and Ikpikpuk River follow similar migration routes and use the same stop-over and wintering sites. Thus, the large differences in blood Hg concentrations found between Semipalmated Sandpipers sampled at Utqiaġvik and Ikpikpuk River suggest that breeding location is a main factor influencing exposure. Furthermore, blood Hg concentrations in migratory Semipalmated Sandpipers sampled in Spring 2011 and 2012 at a stopover site (Delaware Bay, NJ) were much lower than those seen in breeding Semipalmated Sandpipers sampled in the current study (means ranged between 0.01 and 0.02 µg/g with a maximum concentration of 0.10, $n = 30$ µg/g; Tsipoura et al. 2017). Additionally, a previous study at East Bay study found a significant increasing relationship between blood Hg concentrations and sampling date (Hargreaves et al. 2011). Thus, the relationship is far from clear and further study is needed.

Many aspects of Hg toxicokinetics in wild birds, particularly long-distance migrants, are poorly understood. A laboratory study on non-migratory Zebra Finches (*Taeniopygia guttata*) indicated that tissue catabolism during flight may result in increased blood Hg concentrations (Seewagen et al. 2016). In that study, the researchers found an average decrease in lean mass of 12% was related to an increase in blood Hg concentrations of over 10%. Long-distance migrants are likely to have much greater lean mass catabolism during migration. Therefore, the negative relationship between blood Hg concentrations and sampling date observed in the current study could be the result of Hg mobilization due to tissue catabolism during migration, potentially resulting in elevated blood Hg concentrations upon arrival at the breeding grounds. However, when the bird was actually captured and sampled within a season could then potentially unravel any effect from this potential mass catabolism. Further research on the effects of long-distance migration on Hg toxicokinetics and blood Hg concentrations in birds may be beneficial for interpreting blood Hg concentrations in migrating birds.

We found a significant, but weak trend indicating that blood Hg concentrations had a negative relationship with body mass in shorebirds sampled in this study. Other studies have characterized body condition (mass controlled by skeletal size, body mass/culmen length), and have found a negative relationship between body condition and Hg exposure for diving ducks and rails sampled in coastal California (Takekawa et al. 2002, Ackerman et al. 2012). However, previous work on shorebirds at East Bay found no relationship between blood Hg concentrations and body condition (as determined by mass controlled by skeletal size, body mass/culmen length; Hargreaves et al. 2010). In the current study, we were not able to ascertain the underlying cause for the observed relationship between blood Hg and body mass. There may be a causal effect of Hg exposure on body mass, however, previous research found reduced blood Hg concentrations as body mass increased in growing chicks (Ackerman et al. 2011), though this dilution effect has not been documented in adult birds. Sex could be an additional confounding factor, as female shorebirds are generally larger than males and had lower blood Hg concentrations. Overall, we suggest that the relationship between blood Hg concentrations and body mass in shorebirds may be complicated by multiple factors and should be further investigated.

Most shorebirds in this study were sampled during the incubation period when females had recently completed egg laying. Therefore, the lower blood Hg concentrations found in females were likely due to MeHg deposition in eggs (Robinson et al. 2012). Indeed, previous work found higher concentrations of Hg in shorebird eggs in Alaska as compared to most other locations (Saalfeld et al. 2016), and much of our data comes from Alaska. Lower blood Hg concentrations in females may also help explain the inverse relationship between blood Hg and body mass, given that female shorebirds are generally larger than males. A previous study found male Dunlin breeding in Alaska had higher blood Hg concentrations compared to females, but no difference in concentrations was found between sexes of Semipalmated Sandpipers (Perkins et al. 2016). Blood Hg concentrations in breeding shorebirds at East Bay did not differ significantly by sex, but mean Hg concentrations were higher in male Black-bellied Plovers and Ruddy Turnstones, though not for Semipalmated Plovers (*Charadrius semipalmatus*; Hargreaves et al. 2010). The proportion of MeHg that females deposit into eggs can differ by species and with increasing Hg exposure (Robinson et al. 2012, Ackerman et al. 2016b), however, we found similar differences in blood Hg concentrations between males and females for all species included in this analysis. This research further indicates that sex is an important factor influencing blood Hg concentrations and should be considered when using birds as bioindicators of Hg risk in a system.

Blood Hg concentrations have been shown to quickly decrease during feather molt (Bearhop et al. 2000, Bottini et al. 2021). Dunlin were the only species undergoing flight feather molt during this study, and the extent of flight feather molt was recorded for Dunlin sampled at the

Utqiaġvik and Colville River sites. We examined the influence of flight feather molt on blood Hg concentrations but did not find a significant relationship. In general, Dunlin had low blood Hg concentrations at both study sites, which may reflect the fact that Hg was being shunted into flight feathers. Closer examination of the relationship between flight feather molt and blood Hg concentrations in birds with a broader range of Hg exposure would aid interpretation of our study.

Feather Hg concentrations in shorebirds

This study aimed to sample shorebird feathers that were likely grown on the wintering grounds. Overall, shorebirds sampled in this study had higher feather Hg concentrations than blood Hg concentrations, with elevated feather concentrations seen in some individuals. Perkins et al. (2016) determined feather Hg concentrations for breeding Semipalmated Sandpipers and Red Phalaropes at Utqiaġvik in 2008 were ~20% greater than in the current study, and Hargreaves et al. (2010, 2011) reported higher mean feather Hg concentrations at East Bay in 2008 and 2009 in Ruddy Turnstones (41–63% greater) and Red Phalaropes (23% greater). However, mean feather Hg concentrations in Black-bellied Plover varied widely among years with no trend between the earlier and the current study periods. Overall, comparisons with previous studies indicated variable feather Hg concentrations in Arctic-breeding shorebirds sampled at these two study sites.

We did not see a relationship between feather Hg concentrations and sampling sites, as expected if feather Hg concentrations are reflective of winter instead of breeding ground exposure. An example of this may be seen for the phalarope species, both of which had among the lowest feather Hg concentrations in this study. These species are pelagic during the non-breeding season and forage within a low trophic level, planktivorous marine food web (Rubega et al. 2000, Tracy et al. 2002). Therefore, they likely have low Hg exposure during winter, which was reflected in their feather Hg concentrations.

We found little influence of wintering region on body feather Hg concentrations between Dunlin subspecies. While mean feather Hg concentrations were slightly greater for *arcticola*, there was no significant relationship between subspecies and feather Hg concentrations. This result was unexpected, given the predicted higher levels of Hg exposure in East Asia where *arcticola* winter, compared to the coast of North America where *pacifica* winter (Gill et al. 2013). It is possible that our assumption that black breast feathers were grown on the wintering grounds was not always met, as it is not uncommon to see *arcticola* Dunlin arrive in the Arctic with only partially black breasts (R. Lanctot, *unpublished data*). Therefore, some of the breast feathers collected in this study when adults were captured at nests may have been grown on the breeding grounds, confounding any difference between the two subspecies in Hg exposure.

We investigated the influence of sex on feather Hg concentrations for a subset of species and found males had higher feather Hg concentrations than females in most species. The difference between sexes varied among species and Long-billed Dowitcher females had higher feather Hg concentrations than males. A previous study at the East Bay site did not find sex differences in feather Hg concentrations in Black-bellied Plovers, Ruddy Turnstones, or Semipalmated Plovers (Hargreaves et al. 2010). Feather Hg concentrations in Dunlin (*C. a. pacifica*) sampled in British Columbia, Canada, prior to breeding also did not differ by sex (St. Clair et al. 2015). Therefore, we had no general reason to expect higher feather Hg concentrations for either sex, with the exception of the Western Sandpiper which is sexually segregated on the wintering grounds with males wintering in more northern and industrialized areas (Nebel et al. 2002) where Hg exposure might be greater. Higher feather Hg concentrations found for male Western Sandpipers in this study indicate that this may be the case.

We further investigated if bill length sexual dimorphism could result in species differences in non-breeding season Hg exposure. A priori, we expect species with little bill dimorphism to have similar winter diets, and thus similar feather Hg concentrations.

We found Long-billed Dowitchers and Western Sandpipers had the greatest difference in bill length by sex among the species studied, and thus likely the largest wintering dietary differences. These species also showed the largest differences in feather Hg concentrations between males and females (Fig. 6; Supplementary Materials 2). Despite somewhat different bill lengths between male and female Dunlin, little difference in feather Hg concentration was found in this study or by St. Clair et al. (2015). Male Dowitchers, female Dunlin, and female Western Sandpipers have similar bill lengths and similar feather Hg concentrations, consistent with similar bill-length driven exposure and Hg incorporation into feathers. Females have longer bills than males in all five species and had lower feather Hg concentrations than males for all species, except Long-billed Dowitcher. This indicates that, in general, the sex-bias towards surface feeding versus probing deeper in intertidal substrates may have resulted in higher Hg exposure, contrary to previous studies indicating invertebrates located deeper in sediments had greater MeHg concentrations (Sizmur et al. 2013). Our results indicates that further research is needed to better understand sex differences in Hg exposure during the non-breeding season.

Shorebird health risks due to Hg exposure

While the risk categories predicting adverse health effects of Hg in birds were not specifically developed for shorebirds, they are currently the best available benchmarks for adverse health effects of Hg exposure for the birds sampled in this study. We aimed to improve these risk categories by including an additional category specifically based on adverse effects (reduced reproduction) seen in a small avian songbird invertivore (Jackson et al. 2011). Overall, Arctic-breeding shorebirds sampled in this study had blood Hg concentrations similar to background and low risk of adverse effects of Hg exposure. However, the majority of individuals at greater risk for Hg exposure were sampled at Utqiaġvik, indicating that this site may expose shorebirds to Hg for some unknown reason. Further, we documented that certain species, such as Long-billed Dowitchers and Pectoral Sandpipers, may be at greater risk of adverse effects of Hg exposure compared to other species. Note, however, that the potential adverse effects of Hg exposure could be minimized by other elements, such as selenium, that can be found at high levels in Arctic-breeding shorebirds (Saalfeld et al. 2016), as selenium has been shown to counteract the toxicity of Hg (Stoewsand et al. 1974, Khan and Wang 2009).

Limited data are available for toxicity benchmarks for Hg exposure based on feather Hg concentrations. Here, we developed risk categories based on adverse effect thresholds determined for a single species of avian songbird invertivore (Jackson et al. 2011) and we sampled different feather types, making comparisons problematic. However, similar to our results for blood Hg concentrations, we found most shorebirds sampled in this study exhibited a low risk of adverse effects of Hg exposure, based on feather Hg concentrations.

Conclusion

This study investigated factors influencing Hg exposure in Arctic-breeding shorebirds by utilizing a large-scale collaborative network to simultaneously collect nearly 2500 blood and feather samples. This work is timely given the Minamata Convention on Mercury which entered into force in 2017 as a global legally binding agreement to protect the environment and human health from the adverse effects of mercury pollution. Within the Convention, Article 22 focuses on effectiveness evaluation which will likely involve the use of biomonitoring data from biota (https://mercuryconvention.org/sites/default/files/documents/information_document/4_INF12_MonitoringGuidance.English.pdf). The broad dataset from our study provides baseline measurements that can aid in evaluating changes in Hg emissions over time. Given conservation concerns over Arctic-breeding shorebirds, this work also provides information on potential risks due to Hg exposure. In general, based on our findings, we conclude that Arctic-breeding shorebirds were at low risk of adverse effects of Hg exposure. However, blood Hg concentrations were influenced by species and site, and birds sampled at Utqiaġvik were at the greatest risk for adverse effects of Hg exposure. Differences among species indicated that some species were at greater risk of adverse effects than others. While blood Hg concentrations were influenced by foraging habitat moisture classification for some study sites, no clear trend was determined. We found blood Hg concentrations were also influenced by sex, body mass, and capture day. Feather Hg concentrations, reflective of non-breeding habitat, were also influenced by species and sex, with males generally having higher Hg concentrations than females. Our research indicates that many factors influence blood and feather Hg concentrations, therefore, care should be taken when using birds as bioindicators of Hg risk within a system. Including multiple species from the same foraging guild and ecoregion in Hg exposure assessments may better highlight regions of greatest Hg exposure, especially when testing blood since this will reflect local conditions and the inter-species comparisons will not be confounded by wintering region. Understanding the relative influence of multiple factors on Hg concentrations in a group of terrestrial invertivores, as done in this study, can aid in the interpretation of how these concentrations relate to environmental Hg contamination for future studies.

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Author contributions MP, NB, IS, RL, and SB contributed to the study conception and design. JB, MB, JC, WE, RG, GG, M-AG, KG, BH, EK, J-FL, DBL, NL, DP, JR, KR, MR, SS, FS, PS, BW, and PW supervised or led all fieldwork and collected all samples. KR analyzed all samples, and MP performed all data analysis. MP, NB, and IS led writing of the manuscript, with notable help from DBL, NL, and SS. All authors read, commented on, and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval For all samples used in this research, capture, handling, and sampling of animals took place under applicable state and federal permits for wildlife research under the Biodiversity Research Institute (BRI), the U.S. Geological Survey, USFWS, and the Canadian Wildlife Service. All sampling followed protocols approved by the Institutional Animal Care and Use Committees or Animal Use Protocol Approval at the USFWS and all associated universities. Samples from Canadian study sites were shipped to BRI under a USFWS import permit (MB083478). We acknowledge that our work was performed in several Indigenous territories, and we are appreciative of the support received from the communities.

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