The Condor 112(4):789–799

GEOGRAPHIC AND SEASONAL VARIATION IN MERCURY EXPOSURE OF THE DECLINING RUSTY BLACKBIRD

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Abstract. Recent evidence suggests that mercury exposure has negative effects on the health of songbirds, and species that forage in wetlands may be at a greater risk of bioaccumulation of mercury than are those of other habitats. We examined mercury concentrations in blood and feathers from the wetland obligate and rapidly declining Rusty Blackbird (*Euphagus carolinus*) from five regions across North America: three wintering areas in the contiguous United States and breeding areas in the western boreal forests of Alaska and the Acadian forests of northeastern North America. In blood, mercury concentrations in Rusty Blackbirds from the Acadian forest (geometric mean 0.94 μg g^{-1}; $n = 59$) were >3× than in those from Alaska (0.26 μg g^{-1}; 107). Wintering birds had blood mercury levels approximately an order of magnitude lower than those of breeding birds (0.07 μg g^{-1}; 332). In feathers, mercury concentrations in samples from the Acadian forests exceeded published minimum levels for adverse effects on birds (8.26 μg g^{-1}; 45) and were 3× to 7× those observed from the other regions. The mercury concentrations we report in blood and feathers of the Acadian forest population of the Rusty Blackbird are among the highest reported for wild populations of passerines at sites without a known local source of mercury. Mercury should be considered as a potential contributor to the species' dramatic population decline in New England and the Maritime provinces and in other areas where bioavailability of mercury is high.

Key words: *boreal wetlands*, Euphagus carolinus, *icterid*, *mercury*, *methylmercury*, *Rusty Blackbird*.

Variación Geográfica y Estacional en la Exposición al Mercurio de la Especie en Disminución *Euphagus carolinus*

Resumen. Evidencia reciente sugiere que la exposición al mercurio tiene efectos negativos en la salud de las aves canoras, y las especies que se alimentan en humedales pueden estar ante un riesgo mayor de bioacumulación de mercurio que aquellas que viven en otros hábitats. Examinamos concentraciones de mercurio en sangre y plumas en *Euphagus carolinus*, una especie que vive en humedales y cuyas poblaciones se hallan en rápida disminución, en cinco regiones a lo largo de América del Norte: tres áreas de invernada en la parte contigua de Estados Unidos, y en dos áreas de reproducción en los bosques boreales occidentales de Alaska y los bosques Acadios del noreste de América del Norte. Las concentraciones de mercurio en sangre en los individuos de *E. carolinus* del bosque Acadio (media geométrica 0.94 μg g^{-1}; $n = 59$) fueron tres veces mayores que las de los de Alaska (0.26 μg g^{-1}; 107). Las aves invernales mostraron niveles de mercurio en sangre aproximadamente un orden de magnitud menor que los de aves reproductivas (0.07 μg g^{-1}; 332). En las plumas, las concentraciones de mercurio en muestras de los bosques Acadios excedieron los niveles mínimos publicados para efectos adversos (8.26 μg g^{-1}; 45) y fueron de tres a siete veces mayores que las observadas en otras regiones. Las concentraciones de mercurio que informamos en sangre y plumas de las poblaciones de *E. carolinus* del bosque Acadio se encuentran entre las más altas descriptas para poblaciones silvestres de paserinos de sitios sin una fuente local de mercurio conocida. El mercurio debería ser considerado como un colaborador potencial en la disminución drástica de las poblaciones de esta especie en Nueva Inglaterra y en las provincias marítimas de Canadá y en otras áreas donde la bioacumulación de mercurio es alta.

Manuscript received 30 July 2010; accepted 9 September 2010.
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The Condor, Vol. 112, Number 4, pages 789–799. ISSN 0010-5422, electronic ISSN 1938-5422. DOI: 10.1525/cond.2010.100145

INTRODUCTION

The Rusty Blackbird (*Euphagus carolinus*) has experienced a population decline of >85% since the 1960s and, on the basis of the Breeding Bird Survey, may be losing population at a rate of −13% per year, a rate of decline exceeding that of other boreal breeding birds (Greenberg and Droege 1999, Niven et al. 2004, Sauer et al. 2008). Several factors have been proposed as possible drivers for this dramatic population loss, including anthropogenic effects on both breeding and wintering habitat, climate change, and increases in environmental contaminants, but there is no consensus (Greenberg et al. 2011). Environmental mercury (Hg) pollution has been blamed for reduced productivity of birds of both aquatic and terrestrial habitats (Brasso and Cristol 2008, Burgess and Meyer 2008), and Hg has been shown to accumulate in songbirds from a variety of habitats (Evers et al. 2005, Rimmer et al. 2005, Shriver et al. 2006). As the Rusty Blackbird has several characteristics that may predispose it to a high uptake of Hg, and as the mechanism(s) for its population decline is not well understood, we found it prudent to investigate the exposure of this disappearing species to this toxicant.

Heavy metals are well-known toxicants in the environment, and even minimal exposures have been shown to cause sublethal chronic effects in birds (Scheuhammer 1987). Unlike most heavy metals, Hg can undergo both long-distance transport and biomagnification through food chains (Fitzgerald et al. 1998). Hg is present in several forms in the environment, such as Hg(0), Hg(II), and CH_3Hg, with methylmercury (CH_3Hg, hereafter MeHg) being the most toxicologically significant in birds because of its high bioavailability (Heinz 1974, Wolfe et al. 2007). Even in remote locations songbirds can be exposed to Hg through atmospheric deposition (Rimmer et al. 2005), and aquatic point-source contamination can enter adjacent terrestrial food webs (Cristol et al. 2008). High Hg levels in blood and feathers have been correlated with physiological and reproductive effects in an insectivorous songbird (Tree Swallow, *Tachycineta bicolor*; Brasso and Cristol 2008, Franceschini et al. 2009, Hawley et al. 2009, Wada et al. 2009). Mercury exposure through biomagnification depends upon several factors, including foraging guild, habitat, season, and geographic region (Kidd et al. 1995, Evers et al. 2005, Rimmer et al. 2009). Wildlife is at greater risk for Hg exposure in regions of higher atmospheric Hg deposition, such as in the New England and Canadian Maritime region (Evers et al. 2007), and at sites of point-source contamination (Cristol et al. 2008, Hill et al. 2008).

The Rusty Blackbird breeds almost exclusively in forested wetlands across Canada, Alaska, and northern New England (Avery 1995). It winters primarily in the southeastern U.S. in habitat with shallow standing water (Jaramillo and Burke 1999, Niven et al. 2004). Nonbreeding Rusty Blackbirds may be less selective about habitat use and are sometimes found in drier habitats such as pecan orchards, agricultural fields, and urban areas. While breeding they eat both terrestrial and aquatic invertebrates; during the autumn and winter their diet includes a greater percentage of vegetable matter (primarily seeds and tree mast; Beal 1900, Ellison 1990, Avery 1995). Consumption of organisms high in the food web and foraging in acidic wetlands favorable for Hg methylation could predispose this species to higher MeHg exposure. Our objectives for this study were to determine if Rusty Blackbirds accumulate MeHg to levels that are known to have negative effect on fitness and to determine whether there are geographic or seasonal variations in their MeHg exposure.

METHODS

STUDY SITES

We collected samples from five regions across the range of the Rusty Blackbird (Fig. 1). Breeding-season samples (May–July) came from sites across the Acadian forest ecoregion of New England and the Canadian Maritimes (Maine, New Brunswick, New Hampshire, Nova Scotia, and Vermont) and from sites across the western boreal forests of Alaska. Winter-range samples (December–April) came from three regions of the U.S.: the Atlantic coastal plain (North Carolina, South Carolina, and Virginia), the central Ohio River valley in western and central Kentucky, and the Mississippi Alluvial Valley (Arkansas, Louisiana, and Mississippi).

TISSUE SAMPLING

We sampled blood and/or flight feathers from 579 Rusty Blackbirds for total Hg (includes organic and inorganic forms, hereafter THg) between 2005 and 2010. As Rusty Blackbirds are territorial during the breeding season and gregarious in winter, we used different strategies for sampling on the breeding and wintering grounds. In winter we targeted flocks and acquired many replicate samples from fewer sites. Conversely, we sampled breeding pairs at many different sites with less replication from each site. At all sites, birds were captured in nylon mist nets (3 or 12 × 2.6 m, with 33-, 36-, or 61-mm mesh). Breeding birds were often attracted with audio recordings and captured through the strategic placement of nets and decoys along observed flight paths. Wintering blackbirds were captured in mist nets; net sites were occasionally baited with a mix of boiled eggs and cracked corn set 2 to 4 days prior to attempts to net (described by Mettke-Hofmann et al. 2010). Bait was quickly consumed, and telemetry suggests that individual birds spent little time at the bait stations (<10% of their foraging time, Mettke-Hofmann, unpubl. data). Therefore, we assumed that the effect of the bait on THg concentrations in circulating blood was limited. Individuals were banded with a unique U.S. Geological Survey band.

Blood samples (20 to 70 μL) were collected from the cutaneous ulnar vein in a heparinized capillary tube, stored on ice in a Vacutainer, and frozen within 24 hr until analysis. Blood THg concentrations represent uptake of MeHg during the

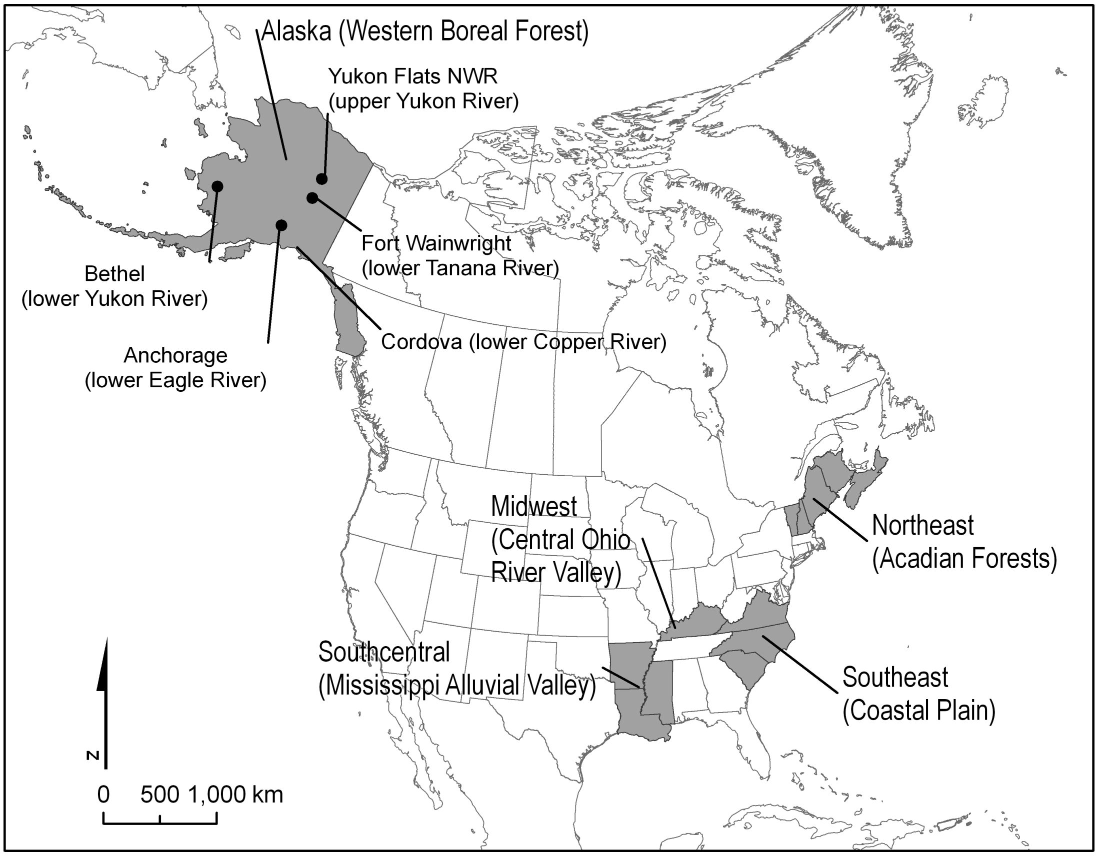

FIGURE 1. Regions and sites serving as sources of Rusty Blackbird tissue for analysis of mercury (Hg).

previous days or weeks (Evers et al. 2005, Hill et al. 2008). One to two flight feathers (first primary or second secondary) were pulled or clipped at the calamus and stored in a plastic envelope until analysis. MeHg is bound to the feather's protein matrix during its active growth, allowing for the sequestration and removal of the contaminant from the body (Dauwe et al. 2003). In the Rusty Blackbird, the annual pre-basic molt of flight and tail feathers follows breeding (Mettke-Hofmann et al. 2010). THg concentrations in flight feathers, therefore, should be similar year round and roughly indicate the burden of MeHg during feather growth in late summer.

LABORATORY ANALYSIS

Using gold-amalgamation atomic absorption spectroscopy with a Milestone DMA-80 or a Nippon MA-2000 direct Hg analyzer, we analyzed blood and feather samples for THg at Acadia University, Nova Scotia; BioDiversity Research Institute, Maine; College of William and Mary, Virginia; and Trace Element Research Lab at Texas A&M University, Texas. Blood samples were thawed, expressed from capillary tubes into combustion boats, and weighed to the nearest 0.1 mg. Feathers were clipped at the calamus and at 1 to 2 cm from the distal end for use in other studies; the remaining central portion of each feather was weighed to the nearest 0.1 mg and placed directly in a combustion boat. THg was determined by EPA method 7473 (U.S. EPA 1998). Internal quality control included initial and continuing verification of calibration, blanks, sample replication, and certified reference materials (DOLT-3, DOLT-4, and/or DORM-3, National Research Council of Canada, Ottawa, Canada) every 10 to 15 samples. The relative percent difference (RPD) for duplicate samples was 4 ± 2% SD ($n = 17$) for blood and 16 ± 20% SD ($n = 44$) for feathers. Mean recovery of certified reference materials was >95% for each batch analyzed with an overall mean recovery of 102 ± 3% SD ($n = 220$).

We analyzed a subsample of five blood and five feathers from 10 individuals for MeHg at Acadia University to confirm that THg in these tissues was representative of MeHg, as demonstrated in previous studies (Evers et al. 2005, Rimmer et al. 2005, Wada et al. 2009). Each sample was weighed to the nearest 0.1 mg, digested in 10 mL of 25% KOH/MeOH, shaken for 1 hr, and placed in a dry bath for 2 hr at 95 °C, methods similar to those of Cai and Bayona (1995). A sample aliquot (20 μL) was transferred to a reaction bubbler and analyzed for MeHg through ethylation and purge-and-trap gas chromatography prior to detection by atomic fluorescence spectrometry

(Brooks Rand Model III) by EPA method 1630 (U.S. EPA 2001). Quality assurance included analytical sample replication (RPD blood = 2 ± 1%; RPD feathers = 0.1 ± 0.05%), internal standards, method blanks, and certified reference material (DOLT-4, mean recovery = 98 ± 4% SD, $n = 3$).

STATISTICAL ANALYSIS

Data were $\log_{10}$-transformed prior to analysis to meet the assumption of normality and normalize the residuals. We used analysis of variance (ANOVA) with Tukey's honestly significant difference (HSD) to separately test for differences by region in the means of THg concentrations in blood and feathers. We used ordinary least-squares regression to separately test the relationship between THg in blood and feathers of breeding and wintering Rusty Blackbirds. Statistical analyses were run in SYSTAT 12.0 (SYSTAT 2007). We used $\alpha = 0.05$ to denote statistically relevant differences in population means. Arithmetic and geometric means are provided for Hg concentrations in µg g^{-1} wet weight for blood and µg g^{-1} fresh weight for feathers, ± SD.

In Alaska we concentrated our sampling at five distinct study areas around the state (Fig. 1): Bethel (lower Yukon River), Anchorage (lower Eagle River), Cordova (lower Copper River), Yukon Flats National Wildlife Refuge (NWR, upper Yukon River), and Fort Wainwright (lower Tanana River). We present THg concentrations in blood for each of these five study areas for subregional comparison of Hg exposure, though sample sizes for some study areas were limited.

RESULTS

We found both geographic and seasonal differences in THg concentrations in blood of Rusty Blackbirds (Fig. 2, 3). Of the five regions, THg concentrations in blood were highest in the Acadian forests, with the single greatest concentration sampled in a male near Kejimkujik National Park in Nova Scotia

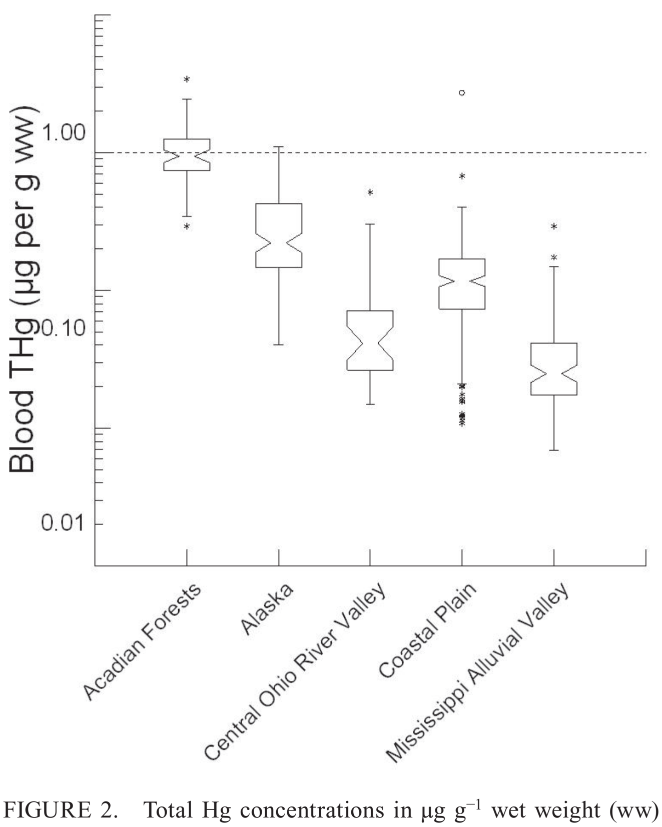

FIGURE 2. Total Hg concentrations in µg g^{-1} wet weight (ww) in blood of the Rusty Blackbird (*Euphagus carolinus*) from five regions of North America on a $\log_{10}$ scale. Boxes are notched at the median and return to full width at the upper and lower limits of confidence intervals; outer limits of the box mark the lower and upper quartiles. Whiskers represent data within 1.5 H-spreads of the interquartile range, asterisks represent outlier values between 1.5 and 3 H-spreads, and circles represent values beyond 3 H-spreads. The horizontal dashed line designates an estimated lower adverse-effects level of 1 µg g^{-1} for THg in Rusty Blackbird blood.

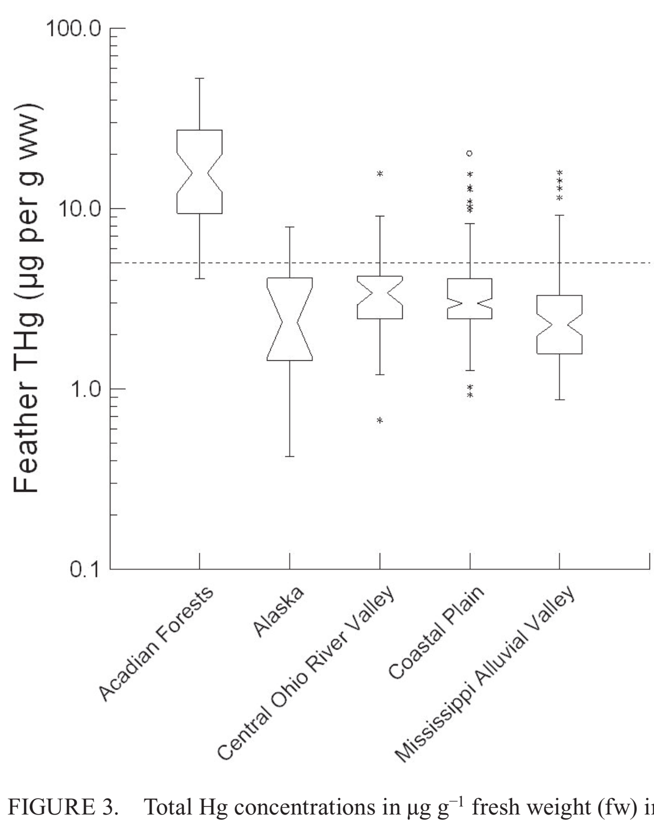

FIGURE 3. Total Hg concentrations in µg g^{-1} fresh weight (fw) in feathers of the Rusty Blackbird (*Euphagus carolinus*) from five regions of North America on a $\log_{10}$ scale. Boxes are notched at the median and return to full width at the upper and lower limits of confidence intervals; outer limits of the box mark the lower and upper quartiles. Whiskers represent data within 1.5 H-spreads of the interquartile range, asterisks represent outlier values between 1.5 and 3 H-spreads, and circles represent values beyond 3 H-spreads. The horizontal dashed line designates the minimum level for adverse effects on birds recommended by Burger and Gochfeld (1997) for THg in feathers.

TABLE 1. Mean concentrations of total Hg in blood (μg g^{-1}, wet weight) and feathers (μg g^{-1} fresh weight) of adult Rusty Blackbirds (*Euphagus carolinus*) by region. Overall means are weighted by regional sample sizes.

Study region or area (season)	Blood: Arithmetic mean ± SD, (*n*); geometric mean	Blood: 95% confidence limits	Feathers: Arithmetic mean ± SD, (*n*); geometric mean	Feathers: 95% confidence limits
Acadian forests (breeding)	1.06 ± 0.54 (59) 0.94	0.92; 1.20	19.57 ± 12.91 (45) 8.26	15.69; 23.46
Alaska: Anchorage (lower Eagle River, breeding)	0.30 ± 0.21 (53) 0.25	0.25; 0.36	—	NA
Alaska: Bethel (lower Yukon River, breeding)	0.15 ± 0.11 (9) 0.14	0.06; 0.23	3.45 ± 2.02 (10) 3.34	2.00; 4.89
Alaska: Cordova (lower Copper River, breeding)	0.18 ± 0.06 (3) 0.18	0.04; 0.33	—	NA
Alaska: Fort Wainwright (lower Tanana River, breeding)	0.59 ± 0.24 (25) 0.51	0.46; 0.66	2.04 ± 1.65 (3) 1.06	−2.05; 6.14
Alaska: Yukon NWR (upper Yukon River, breeding)	0.17 ± 0.12 (25) 0.15	0.12; 0.22	0.42 (1) 0.42	NA
Alaska (breeding)	0.31 ± 0.24 (115) 0.25	0.27; 0.36	2.93 ± 2.03 (14) 1.11	1.76; 4.10
Atlantic coastal plains (wintering)	0.14 ± 0.19 (209) 0.10	0.11; 0.16	3.83 ± 2.95 (147) 2.29	3.35; 4.31
Central Ohio River valley (wintering)	0.07 ± 0.10 (31) 0.05	0.04; 0.11	3.98 ± 2.86 (31) 3.30	2.93; 5.02
Mississippi Alluvial Valley (wintering)	0.04 ± 0.04 (92) 0.03	0.03; 0.05	3.24 ± 3.06 (72) 2.04	2.52; 3.96
Overall breeding	0.57 ± 0.51 (174) 0.39	0.49; 0.64	15.62 ± 13.35 (59) 2.19	12.14; 19.10
Overall wintering	0.10 ± 0.17 (332) 0.07	0.09; 0.12	3.68 ± 2.97 (250) 2.30	3.31; 4.05
Overall mean	0.26 ± 0.39 (506) 0.12	0.23; 0.30	5.96 ± 7.93 (309) 2.26	5.07; 6.85

(3.42 μg g^{-1} wet weight, Table 1, $F_{4,501} = 261.5$, $P < 0.001$). THg concentrations in blood were significantly higher in both regions of the breeding range examined than in either region of the winter range, being at least 7× greater in the Acadian forests than in either region of wintering (Table 2).

Within Alaska, a comparison of 95% confidence limits for THg concentrations in blood suggests differences by subregion (Table 1). The greatest THg concentrations in blood were found along the lower Tanana River at Fort Wainwright, with the maximum value for Alaska from a female sampled at this site (1.11 μg g^{-1}).

THg concentrations in feathers from the Acadian forests differed from those of the other four regions (Table 2, $F_{4,304} = 78.1$, $P < 0.001$), being 3× to 7× greater than those observed elsewhere (Table 1, 2). Other regions did not differ significantly, except between the coastal plains and Mississippi Alluvial

TABLE 2. Tukey's pair-wise comparison of total Hg concentrations in blood ($F_{4,501} = 261.5$; $P < 0.001$) and feathers ($F_{4,304} = 78.1$; $P < 0.001$) of the Rusty Blackbird (*Euphagus carolinus*) by region.

Regional comparison with direction of difference	Blood: Difference between least-squares means	Blood: Magnitude of difference between geometric means	Feathers: Difference between least-squares means	Feathers: Magnitude of difference between geometric means
Acadian forests > Alaska	0.59[a]	4×	0.85[a]	7×
Acadian forests > Atlantic coastal plains	0.97[a]	7×	0.69[a]	4×
Acadian forests > central Ohio River valley	1.31[a]	19×	0.68[a]	3×
Acadian forests > Mississippi Alluvial Valley	1.53[a]	31×	0.81[a]	4×
Alaska > Atlantic coastal plains	0.38[a]	3×	−0.16[c]	NA
Alaska > central Ohio River valley	0.72[a]	5×	−0.16[c]	NA
Alaska > Mississippi Alluvial Valley	0.95[a]	9×	−0.04[c]	NA
Atlantic coastal plains > central Ohio River valley	0.34[a]	2×	−0.01[c]	NA
Atlantic coastal plains > Mississippi Alluvial Valley	0.57[a]	3×	0.12[b]	1×
Central Ohio River valley > Mississippi Alluvial Valley	0.23[a]	2×	0.12[c]	NA

[a] $P < 0.05$
[b] $P < 0.1$
[c] $P > 0.1$

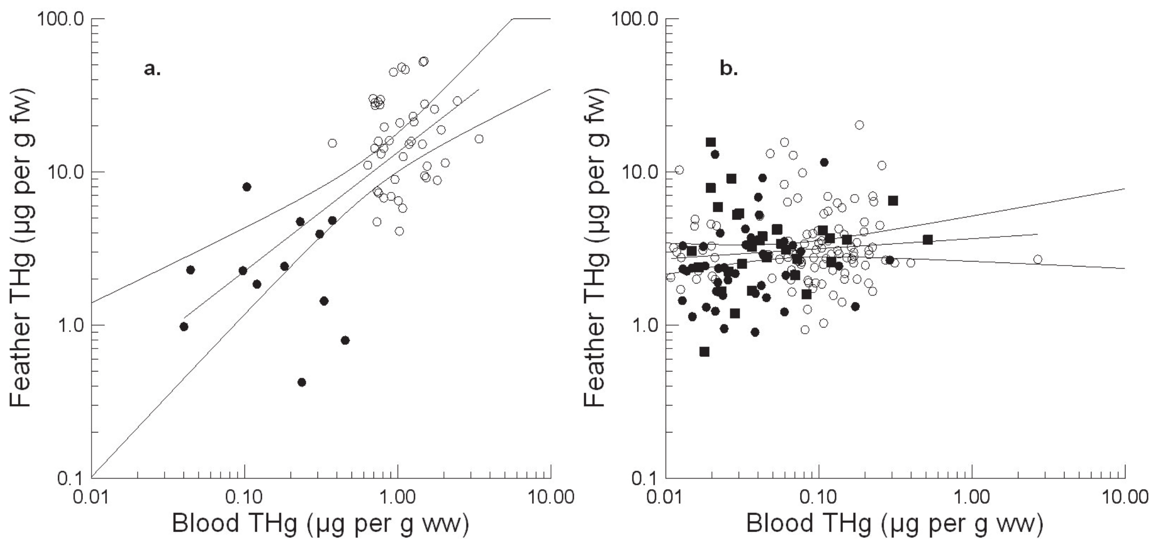

FIGURE 4. Relationship between THg concentrations (µg g^{-1}) in blood and feathers of breeding (a, $R^2 = 0.44$) and wintering (b, $R^2 = 0.02$) Rusty Blackbirds. Circles, birds breeding in the Acadian forest (a) or wintering on the Atlantic coastal plains (b); solid circles, birds breeding in Alaska (a) or wintering in the Mississippi Alluvial Valley (b); solid squares, birds wintering in the central Ohio River valley (b). Center line is linear line of best fit; outer lines mark the upper and lower limits of the 95% confidence interval.

Valley, which differed minimally. We recorded the highest THg concentrations (52 µg g^{-1} fresh weight) from two males, one from northeastern Vermont near Maidstone, the other from northern Nova Scotia near Antigonish.

Mean percent MeHg of THg was 98 ± 2% ($n = 5$) in blood and 97 ± 0.3% ($n = 5$) in feathers. The relationship between THg concentrations in blood and feathers of individual breeding Rusty Blackbirds was significant and positive ($n = 57$, $F_{1,55} = 42.6$, $R^2 = 0.44$, $P < 0.001$, Fig. 4). For wintering Rusty Blackbirds, the relationship was positive but not significant ($n = 179$, $F_{1,177} = 2.95$, $R^2 = 0.02$, $P = 0.09$).

DISCUSSION

MERCURY EXPOSURE IN THE RUSTY BLACKBIRD

The concentrations of THg in blood and feather tissue sampled from Rusty Blackbirds breeding in the Acadian forests were significantly greater than those from the other four regions sampled. Our current understanding of Rusty Blackbird migration suggests an east–west divide along the Appalachian Mountains that corresponds to a population division in the eastern boreal breeding range, a pattern currently considered unique to this species, as the division in the breeding range is not centered along a natural physiographic feature (Hobson et al. 2010). Accordingly, we predicted THg concentrations in feathers from the regions of the south-central U.S. examined in this study (Mississippi Alluvial Valley and central Ohio River valley) to reflect Hg exposures from the western boreal region and those from the southeastern region (coastal plains) to reflect Hg exposures from the eastern boreal region. We detected little to no difference in THg concentrations in feathers among the winter regions, implying similar exposures across the breeding range. Yet we found that THg concentrations in feathers from the Acadian forests were 3× to 4× greater than in those from the winter range, suggesting that Hg exposure is higher in the far eastern portion of the breeding range within the Acadian forests than in the rest of the boreal forest. Sampling of the breeding population across the boreal forest is necessary to clarify this result.

The Hg in blood and feathers was nearly 100% MeHg, as found in other species of birds (Thompson and Furness 1989, Evers et al. 2005, Rimmer et al. 2005, Wada et al. 2009). Thus our use of the more abundant THg data as a proxy for the presence of the highly bioavailable MeHg was valid. Additionally, the THg concentration in feathers was more tightly related to the THg concentration in the blood of breeding birds than it was to that in the blood of wintering birds, consistent with the idea that Rusty Blackbirds accumulate much of their Hg burden during the summer period between the arrival on breeding grounds and the onset of the pre-basic molt. Sampling of diet items throughout the year will be necessary to identify timing and sources of Hg exposures.

THg concentrations in blood generally reflect exposure from the diet during the previous few days or weeks (Evers et al. 2005, Hill et al. 2008). Consequently, the elevated THg concentrations in the blood of breeding birds, particularly in the Acadian forests, likely reflected local Hg exposure. Elevated Hg concentrations in the Acadian forests have been found in other wildlife with continent-wide distributions, such as the Common Loon (*Gavia immer*; Evers et al. 2003). These elevated concentrations are likely due to biogeochemical conditions conducive to methylation in a region with a higher rate of Hg deposition and retention (O'Driscoll et al. 2005). Northeastern North America has historically received the downwind transport of industrial emissions from the Ohio River valley, the mid-Atlantic states, and elsewhere, consequently increasing the Hg levels observed in the environment (Evers and Clair 2005, Keeler et al. 2005). Accordingly, five biological hotspots for Hg exposure have been identified in the region, including the area in and around Kejimkujik National Park in Nova Scotia (Evers et al. 2007).

Habitat type is important in determining Hg availability to a species (Evers et al. 2005), and recent research suggests that microhabitat plays a critical role as well (Eagles-Smith et al. 2009). In general, the wetlands in the Acadian forests that typify the Rusty Blackbird's breeding habitat are characterized by high levels of dissolved organic carbon (average 9.23 mg L^{-1}) and low pH (average 5.78, $n = 21$, Edmonds, unpubl. data). High levels of dissolved organic carbon and low pH have both been correlated with increased methylation, bioavailability, and retention of Hg (Scheuhammer 1991, O'Driscoll et al. 2005, 2006, Harding et al. 2006).

The higher THg levels observed in the blood of breeding blackbirds could also reflect a seasonal trophic shift. Beal (1900) found that the stomachs of wintering Rusty Blackbirds contained primarily vegetable matter, including acorns, corn, and weed seeds, whereas those of breeding birds contained largely animal matter; four Rusty Blackbird stomachs collected in August in Nova Scotia contained 97% insects and spiders. Anecdotally, we observed this change from a diet of insects in the breeding season to a broader diet in winter during our study. In winter, in addition to observing Rusty Blackbirds occasionally foraging for invertebrates and small fish, we often observed them feeding on crushed pecans, acorns, and waste grain. In contrast, prey collected from captured breeding adults included larvae of Diptera (flies), nymphs of Odonata (dragonflies), and larvae of Trichoptera (caddisflies), and we observed territorial birds foraging for emerging adult Odonata and Ephemeroptera (mayflies; Edmonds, Newell, and Tessler, unpubl. data).

While THg levels in the blood of breeding Rusty Blackbirds in Alaska were lower than those found in the Acadian forests, they were higher than those in the winter range and varied within Alaska. The seasonal change of Hg concentrations may partially be explained by a change in diet, as suggested earlier. However, as the breeding diet is likely similar across Alaska, the variation observed within that region suggests that local factors are also important in determining Hg burdens. Some of the observed differences among sites may be due to (1) differences in soil and water quality that enhance Hg methylation (e.g., dissolved organic carbon and pH), (2) point-source Hg contamination, such as historic gold mining in some watersheds, (3) the addition of Hg to some watersheds via melting permafrost (Klaminder et al. 2008), or (4) differences among locales in the length of the food chain or the blackbird's diet. Determining the magnitude and underlying cause(s) of these apparent spatial patterns of Hg in Rusty Blackbirds in Alaska will require continued sampling.

COMPARISON OF MERCURY VALUES IN RUSTY AND RED-WINGED BLACKBIRDS

To provide a context for interpreting the elevated Hg levels we observed in Rusty Blackbirds breeding in northeastern North America, we obtained unpublished data on THg in blood and feathers collected from breeding Red-winged Blackbirds (*Agelaius phoeniceus*) in the same region. The Red-winged and Rusty Blackbirds, both icterids, overlap broadly in range, habitat, diet (Jaramillo and Burke 1999), and pattern of flight-feather molt (Pyle 1997). We queried data on Hg in blood and feathers of the Red-winged Blackbird from MercNet, a comprehensive national Hg database representing available government data that has been compiled and maintained by the BioDiversity Research Institute (Gorham, Maine). We bounded our query by filtering for region and habitat and separating point from nonpoint sources of Hg (date of query 5 September 2009). The query yielded 59 Red-winged Blackbirds sampled on breeding grounds in the Northeast (New York, New England, and the Maritimes) and captured in wetland habitat without a known point source for Hg pollution between May and August from 2003 to 2009 (Table 3). An additional 21 samples were collected between 2003 and 2006 from wetlands along the Sudbury River in Massachusetts that were contaminated by industrial Hg (Wiener and Shields 2000). We collected blood samples and analyzed them for THg by the same protocol as for the Rusty Blackbird; untrimmed flight feathers were collected and analyzed by the same protocol as for the Rusty Blackbird.

THg concentrations in the blood of Rusty Blackbirds breeding in the Acadian forest were approximately 4× the

TABLE 3. Total Hg concentrations (μg g^{-1}) in blood and feathers of adult Red-winged Blackbirds (*Agelaius phoeniceus*) breeding in wetlands, queried from MercNet database, BioDiversity Research Institute (Gorham, Maine, 5 September 2009).

	Source	
Tissue	New York, New England, Maritime provinces	Industrially contaminated wetlands, Sudbury River, Massachusetts
Blood		
Arithmetic mean ± SD (*n*)	0.39 ± 0.31 (50)	1.88 ± 2.97 (20)
Geometric mean	0.22	0.53
95% confidence limits	0.30; 0.48	0.48; 3.26
Feathers		
Arithmetic mean ± SD (*n*)	1.08 ± 2.58 (40)	0.82 ± 0.62 (18)
Geometric mean	0.48	0.65
95% confidence limits	0.26; 1.91	0.51; 1.13

geometric mean of those of breeding Red-winged Blackbirds from uncontaminated sites and twice those of Red-winged Blackbirds from the contaminated site (Table 1, 3). THg concentrations in feathers of the Rusty Blackbird were 17× higher than in those of breeding Red-winged Blackbirds away from a point source and 13× higher than in those of Red-winged Blackbirds from the contaminated site.

We suspect that the differences in Hg concentrations between the two species reflect differences in diet, the type of wetland the species uses, or both. Breeding Rusty Blackbirds are suspected to feed at a trophic level higher than do breeding Red-winged Blackbirds, possibly feeding more frequently on the more predaceous invertebrate taxa Odonata, Diptera, and Arachnida (Beal 1900, Beecher 1951, Ellison 1990, Avery 1995, Yasukawa and Searcy 1995). This suspicion of differences in trophic position requires confirmation through stable-isotope analysis. Shriver et al. (2006) speculated that a nearly two-fold difference in Hg concentration between the closely related and co-occurring Saltmarsh Sparrow (*Ammodramus caudacutus*) and Nelson's Sparrow (*A. nelsoni*) was due to dietary differences. Simultaneous sampling from sites of co-occurrence will be necessary to confirm and explain the higher levels of Hg found in Rusty Blackbirds.

METHYLMERCURY TOXICITY AND POTENTIAL FOR POPULATION-LEVEL EFFECTS

MeHg is an extremely potent neurotoxicant, as well as an immunotoxicant and genotoxicant, with negative effects on reproduction being one of the most sensitive endpoints (Wolfe et al. 1998, 2007). Decreased reproductive success from MeHg exposure has been observed in both laboratory and field studies, and toxicity can result in decreased egg volume and embryo weight, shell thinning, abnormalities and deformities of embryos or hatchlings, and deleterious behavioral changes in adults (Scheuhammer et al. 2007, Evers et al. 2008). Though some field studies of Hg exposure of birds have failed to detect negative effects on health (Ackerman et al. 2008), recent field research on insectivorous Tree Swallows at Hg-contaminated sites indicates that Hg exposure alters a bird's immune responsiveness (Hawley et al. 2009) and thyroid hormone and corticosterone profiles (Franceschini et al. 2009, Wada et al. 2009).

While little research has focused on the effect of MeHg on blackbirds, a recent experimental study by Heinz et al. (2009) suggests that passerines, particularly blackbirds, may be more sensitive to MeHg than are the waterbirds that have received the bulk of study. Of 26 bird species studied, Heinz et al. (2009) considered the two passerines, the Tree Swallow (Hirundinidae) and Common Grackle (*Quiscalus quiscula,* Icteridae), to be moderately sensitive, and the grackle was more sensitive than the Tree Swallow, having a 100% lethal concentration (LC_{100}) for eggs of 0.8 μg g^{-1} compared with 1.6 μg g^{-1} for swallow eggs (Heinz et al. 2009). Similarly, Finley et al. (1979) found that the Common Grackle—a species more closely related to the Rusty Blackbird than the Red-winged Blackbird (Lanyon and Omland 1999)—had the lowest tolerance to lethal MeHg poisoning of three passerines, including the Red-winged Blackbird.

Unfortunately, the egg-dosing study of Heinz et al. (2009) is not directly analogous to Hg deposition by a female into her egg; however, the study does suggest that blackbirds may be more sensitive during embryonic development than previously studied species such as waterfowl. There is currently no basis in the literature for setting a level of concern for Hg in Rusty Blackbird blood, though some bounds can be placed on this number. Evers et al. (2008) detected reproductive effects in Common Loons with 3 μg g^{-1} THg in blood, and Heinz et al. (2009) demonstrated that Common Grackle embryos are more sensitive to injected MeHg than are those of many water birds. For example, at a concentration that killed half of the Mallard (*Anas platyrhynchos*) embryos (1.8 μg g^{-1}), 100% of the Common Grackle embryos were dead (Heinz et al. 2009). Thus it seems reasonable to assume that for blackbirds adverse effects may occur between 1 and 3 μg g^{-1}, and it would be prudent to assume that the lowest observed adverse-effect level may be lower than 1 μg g^{-1}. In the Rusty Blackbirds sampled for this study from the Acadian forest, THg concentrations exceeded 1 μg g^{-1} in 47%, suggesting that there is reason for concern regarding the THg levels observed in the blood of this declining species (Fig. 2).

Reduced hatching of eggs has been documented when THg concentrations in feathers are between 5 and 40 μg g^{-1} (Burger and Gochfeld 1997). Provided that the feather samples from the winter range fairly represent the Rusty Blackbird's exposure before molt across the boreal forest, a relatively low percentage of Rusty Blackbirds may be at risk of negative effects from Hg (16%, Fig. 3). Feathers of over 95% of the birds sampled from the Acadian forests, however, exceeded this threshold.

Although THg concentrations in breeding Rusty Blackbirds were elevated relative to what has been reported in the literature for other passerines away from contaminated sites, and elevated compared to our unpublished sample of Red-winged Blackbirds, there is currently no direct evidence for population-level effects on the Rusty Blackbird resulting from elevated Hg. As Rusty Blackbirds in the far eastern portion of the breeding range had higher levels of Hg than did those in other regions, it is worth asking whether this subpopulation has suffered a more dramatic population decline than those of other regions. Powell (2008) reported a range contraction in Maine of 46% since 1983 despite the apparently suitable habitat, while Breeding Bird Survey data suggest a decline of −10% per year in the East (Sauer et al. 2008). The population decline does not appear to be as dramatic in Alaska, where Breeding Bird Surveys imply a nonsignificant annual population decline of −5% (Sauer et al. 2008). Additionally, Machtans et al. (2007) found no evidence of a population change in the

Mackenzie River Valley, which is in the western boreal forest and near our interior Alaska study sites with lower Hg levels. Exposure to MeHg has been linked with reduced productivity in the Common Loon (Burgess and Meyer 2008) and has been proposed as a factor potentially impeding the recovery of populations of wading birds in southern Florida (Spalding et al. 1994, Sundlof et al. 1994). Effects of MeHg exposure at the level of the individual should be considered among the possible explanations for the declines observed in the far eastern portion of the Rusty Blackbird's breeding range. Nevertheless, other stressors, such as habitat destruction (e.g., Powell et al. 2010), climate change, and additional contaminants may also have an important effect on the eastern population and should be investigated.

SUGGESTIONS FOR FUTURE RESEARCH

Future research should further quantify Hg burdens in breeding Rusty Blackbirds. Additional effort should include other parts of the eastern breeding range such as Quebec, Ontario, and New York, where elevated environmental Hg loads in other birds such as the Common Loon have been documented. More robust data sets are required to explain the spatial variation of THg in the blood of Rusty Blackbirds breeding in Alaska. Continued and standardized continental sampling efforts will also help to measure changes in environmental Hg loads related to projected emission regulations in the U.S., Canada, and potentially elsewhere.

Future research should aim to clarify the role that Hg and other environmental contaminants play in the continuing population decline of the Rusty Blackbird. Researchers should also seek to test the hypothesis that Hg contamination is contributing to the species' population decline through examining individual- and population-level effects on reproduction and survival. As the limited data available suggest the Rusty Blackbird enjoys high rates of hatching success (Powell 2008), we suggest studying not only nesting success but also juveniles' survival rates, as Hg exposure may have increased developmental and survival risks after fledging (Spalding et al. 2000, Condon and Cristol 2009). We further suggest greater sampling of species with stable or increasing populations within the same wetland complexes for direct comparisons of Hg exposures.

ACKNOWLEDGMENTS

We thank the many field biologists and technicians who collected samples while conducting their own research, including Bud Johnson, Dave Loomis, Steve Matsuoka, Dave Shaw, and April Scurr, who provided samples from Alaska. Russ Greenberg, S. Matsuoka, Anna Redden, and Claire W. V. Ramos, along with two anonymous reviewers provided feedback on drafts of this paper. Portions of this research were originally presented to and received useful feedback from the International Rusty Blackbird Technical Working Group at Powdermill Nature Preserve, Rector, Pennsylvania, in October 2008. Locations at which Rusty Blackbird might breed were provided by Caleb Fisher for Vermont, by Carol Foss for New Hampshire, and by the Maritime Breeding Bird Atlas for New Brunswick and Nova Scotia. Melissa Duron assisted with the database query. Housing and logistical support were provided by (in alphabetical order) the Center for Bottomland Hardwoods, Chugach National Forest, Cordova Ranger District, Elmendorf Air Force Base, Fort Richardson, Fort Wainwright, K.C. Irving Center of Acadia University, Theodore Roosevelt NWR Complex, U.S. Forest Service, and Yukon Flats NWR. Brainard Palmer-Ball, Cleaton Baptist Church, the Delta Research and Extension Center, Delta National Forest, Leroy Percy State Park, Silvio O. Conte NWR, Theodore Roosevelt NWR Complex, Vermont Department of Fish and Wildlife, Wagner Forest Management Ltd., Frederick Ballard, and others kindly permitted access to their property. Funding was provided in part by the Alaska Bird Observatory, Alaska Department of Fish and Game State Wildlife Grant Program, the Arthur-von-Gwinner Foundation, Canada Foundation for Innovation, Canada Research Chairs Program, Canadian Wildlife Service, Chugach National Forest, the Department of Defense Legacy Program, Ducks Unlimited Canada, Friends of the National Zoo, German Ethological Society, Kentucky Department of Fish and Wildlife Resources State Wildlife Grant Program, Max Planck Institute for Ornithology of Andechs, National Science and Engineering Research Council of Canada, the Society for Tropical Ornithology, U.S. Fish and Wildlife Service, and the U.S. Geological Survey. We thank Robert Taylor and Deborah Perry at the Department of Veterinary Anatomy and Public Health, Texas A&M University, Trace Element Research Lab, College Station, Texas, for providing Hg analysis during the early years of this study.

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