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Mercury in breeding and wintering Nelson's Sparrows (*Ammodramus nelsoni*)

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Abstract The objective of this study was to increase our understanding of Hg exposure in birds with obligate ties to coastal salt marsh and inland wetland systems. Many species filling such niches are of conservation concern because of reduced size and quality of vital habitats. We used Nelson's Sparrow (*Ammodramus nelsoni*) as an indicator of regional mercury (Hg) availability in its breeding and wintering salt marsh and wetland habitats. Blood, breast feathers and the first primary feather were sampled from Nelson's Sparrows wintering in North Carolina coastal salt marshes and breeding in wetland systems in North Dakota (*A. n. nelsoni*) and Ontario, Canada (*A. n. alterus*). Wintering Nelson's Sparrow breast feathers contained 3.0 times as much Hg as birds breeding in North Dakota and 2.4 times as much Hg as those breeding in Ontario. Breeding Nelson's Sparrows in North Dakota exhibited blood Hg levels 4.9 times as high as those from birds breeding along James Bay and 7.6 times as high as those wintering in North Carolina. These results provide significant insight on the timing of molt in this species as well as how Hg exposure varies regionally and seasonally for these birds. Further, our results provide a better understanding of how and where Hg exposure may be a threat to Nelson's Sparrows and other birds with obligate ties to aquatic systems.

Keywords *Ammodramus nelsoni* · Blood · Feathers · Mercury · Nelson's sparrow · Salt marsh · Wetland

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Introduction

Mercury (Hg) is widely accepted to be one of the most toxic substances in the environment and has been found to negatively impact entire ecosystems (Battaglia et al. 2005; Houserova et al. 2005). Atmospheric mercury is slowly oxidized to Hg^{2+} (mercuric/divalent Hg) and eventually enters aquatic sediments where it can be converted by sulfur-reducing microbes to methylmercury (MeHg) (Clarkson and Magos 2006; Wolfe et al. 1998; Celo et al. 2006). Typical salt marsh hydrology, acid-base status and sediment characteristics result in highly favorable conditions for sulfur-reducing microbial communities, allowing the process of mercury methylation to occur at a rate as much as 25 times higher in marsh habitats than in open water locations (Williams et al. 1994; Marvin-DiPasquale et al. 2003; Gambrell 1994). Factors that affect the accumulation and methylation of Hg in salt marsh and wetland habitats can vary spatially (basin size, land use, soil properties, acid/base status, climate) or temporally (water discharge, water chemistry, redox conditions) (Gambrell 1994; Shanley et al. 2005; Williams et al. 1994), resulting in complex Hg dynamics and the potential for high levels of local variability in these processes.

Nelson's Sparrow (*Ammodramus nelsoni*) is an omnivorous passerine with three subspecies (*A. n. nelsoni*, NSTS-N; *A. n. alterus*, NSTS-A and *A. n. subvirgatus*, NSTS-S) that breed in geographically separate freshwater wetland and salt marsh habitats (Fig. 1) and winter in mixed flocks in salt marshes along the coasts of the southeastern U.S. and Gulf of Mexico. The patchy wetland habitats and limited wintering range of this species have already been reduced and fragmented on the Atlantic seaboard (Greenlaw and Woolfenden 2007) resulting in the recognition of

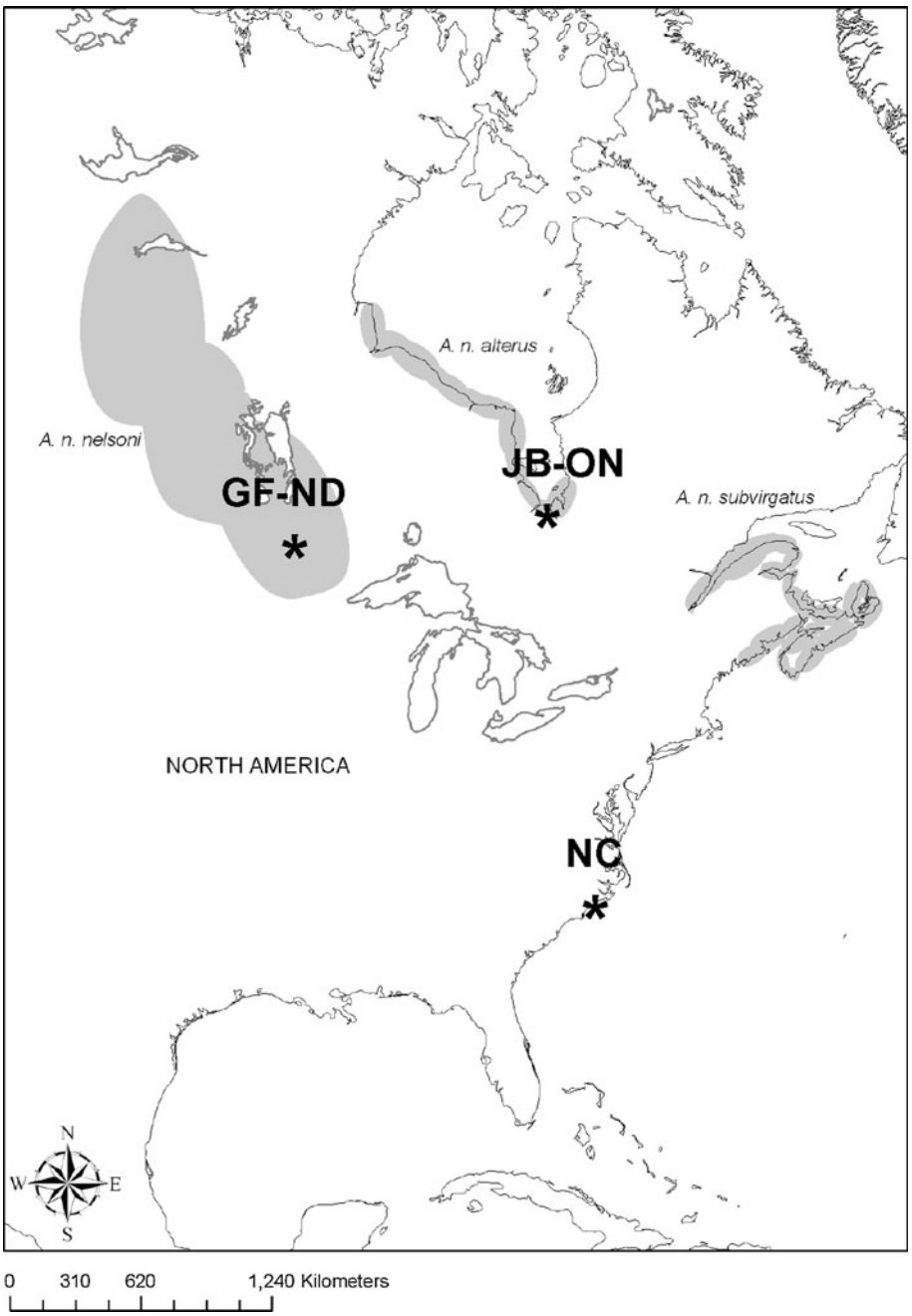

Fig. 1 Map of Nelson's Sparrow breeding ranges (gray) and sampling locations. Subspecies breeding locations are denoted for: *Ammodramus nelsoni nelsoni* (NSTS-N), *A. n. alterus* (NSTS-A) and *A. n. subvirgatus* (NSTS-S). NC represents pooled data from winter captures at three sites near Wrightsville Beach, North Carolina, USA; GF-ND represents breeding captures near Grand Forks, North Dakota, USA (47°54′7.90″ N, 97°17′55.31″ W); JB-ON represents breeding captures from the shore of James Bay North of Moosonee, Ontario, Canada (JB-ON; 51°21′36.53″ N, 80°25′27.79″ W)

Nelson's Sparrow as a species of conservation concern on various watchlists.

Mercury exposure may be an important conservation concern for Nelson's Sparrows since particularly high Hg availability has been reported in areas coinciding with fragmented habitat of this species (Evers et al. 2004; Wolfe et al. 2003). Nelson's Sparrow has been described as a suitable indicator of regional Hg availability in coastal salt marsh and interior freshwater wetland habitats because individuals of this species exhibit obligate ties to these ecosystems throughout their life cycles (Shriver et al. 2006). Nelson's Sparrows are at risk to Hg contamination and bioaccumulation because they: (1) feed at relatively high trophic levels as omnivores, (2) are long-lived and therefore are prone to bioaccumulation, and (3) forage in aquatic environments elevating their risk of exposure to MeHg.

Our study utilizes feathers and blood as tools to examine Hg contamination in breeding and wintering Nelson's Sparrows. Feather Hg reflects dietary uptake immediately prior to molt as well as overall body burden; feathers typically contain ≥90% MeHg regardless of total Hg loads (Furness and Camphuysen 1997; Braune and Gaskin 1987; Bond and Diamond 2009). Feather Hg is also positively

correlated with Hg levels in internal tissues (Lewis and Furness 1993) and typically represents between 70 and 93% of muscle MeHg burden prior to molt (Evers et al. 2005; Braune and Gaskin 1987). Blood Hg has been suggested as the best evaluator of short-term dietary Hg uptake (Evers et al. 2005; Meyer et al. 1998) and typically contains greater than 95% MeHg for both piscivorous (Fournier et al. 2002; Evers et al. 2003) and insectivorous bird species (Rimmer et al. 2005).

In some bird species, Hg levels of 5–40 ppm in feathers and 3.0 ppm in blood have been related to impaired reproduction and subsequent population declines (Evers et al. 2008; Burger and Gochfeld 1997; Brasso and Cristol 2008). However, other species appear to behave and reproduce normally even with feather and blood Hg levels at the high end of or exceeding the ranges described above. For example, research on Bald Eagles (*Haliaeetus leucocephalus*) has indicated that populations with average feather Hg concentrations above 36 ppm exhibit no reproductive or other health effects of Hg bioaccumulation of this magnitude (Bechard et al. 2009). The fact that some species exhibit potentially population-threatening negative effects of Hg exposure at levels far below those observed in other normally functioning, healthy populations demonstrates our lack of understanding of the specifics of Hg toxicity.

The main objective of our study was to assess Hg exposure using relatively understudied populations of breeding and wintering Nelson's Sparrows as indicators of regional Hg availability. Our approach advances the risk assessment of integrated year-round Hg availability in the salt marsh and wetland ecosystems these birds inhabit. In the absence of existing relevant local Hg data for the three regions sampled in this study, we predicted blood Hg levels (reflecting local exposure) would be highest in coastal North Carolina, intermediate in Grand Forks, North Dakota and lowest at James Bay near Moosonee Ontario, corresponding to the varying levels of atmospheric deposition of Hg across these locations (Mercury Deposition Network; http://nadp.sws.uiuc.edu/mdn/). We expected primary and breast feather Hg concentrations to be more indicative of long-term dietary intake over the period between molts; therefore, we anticipated less regional variation in these two tissues compared to blood.

Methods

Study sites and sample collection

To sample wintering populations, Nelson's Sparrows were captured in mist nets (20 mm mesh size) as they became concentrated during spring tides from October 2008 to April 2009 at three comparatively elevated salt marsh islets near Wrightsville Beach, North Carolina, USA. These three sites are Lea-Hutaff (LH, 34°19′45.74″ N, 77°41′30.48″ W), Parnell (P, 34°11′04.69″ N, 77°50′17.74″ W), and Estuarine Reserve (ER, 34°08′17.24″ N, 77°50′48.64″ W). ER is located on the Masonboro Island component of the North Carolina National Estuarine Research Reserve System. Furthermore, the LH site has been designated as an Important Bird Area by Audubon North Carolina, in part because of the presence of coastal sparrow species at this site. Past work on all three of these winter sites has demonstrated that banded Nelson's Sparrows have maintained nearly complete fidelity to their original capture site (Michaelis 2009). Therefore, blood Hg values should reflect contamination on a highly localized scale. During the winter, all three subspecies of Nelson's Sparrow are present in mixed flocks on each of the North Carolina study sites. Identification to subspecies on wintering sites was not always possible due to the considerable overlap in plumage and morphometric characteristics of these groups. Therefore, for the purposes of this study, all North Carolina winter captures were pooled into one group of wintering Nelson's Sparrows referred to hereafter as wintering NSTS.

To sample breeding populations, we used conspecific call playback methods to lure Nelson's Sparrows into mist nets. We captured Nelson's Sparrows in June 2009 at prairie wetland sites within Kelly's Slough National Wildlife Refuge and Grand Forks County Waterfowl Management Areas and Waterfowl Production Areas near Grand Forks, North Dakota, USA (GF-ND; 47°54′7.90″ N, 97°17′55.31″ W; Fig. 1) and hereafter refer to this group as NSTS-N. We also captured Nelson's Sparrows in July 2009 along the shore of James Bay, north of Moosonee, Ontario, Canada (JB-ON; 51°21′36.53″ N, 80°25′27.79″ W; Fig. 1) and hereafter refer to this group as NSTS-A.

Nelson's Sparrows were banded with USGS aluminum bands. Blood was sampled by pricking the brachial vein with a sterile 26G1/2 needle and collecting up to 70 µl using a heparin-coated capillary tube. Capillary tubes were capped with Crito-caps® and stored in plastic vials to prevent breakage. The blood samples were initially stored in a cooler with ice; after returning to the laboratory, samples were stored at −80°C until analysis for Hg content. The first primary feather (P1) was cut using a small pair of scissors as close to the base of the shaft as possible, and eight to ten breast feathers were plucked from each bird and stored in plastic re-sealable bags. P1 was chosen for sampling since it has been documented to have the highest Hg concentration in species that perform a sequential molt and will therefore provide the most consistent and relevant signal of yearly Hg accumulation (Littrel 1991; Braune 1987; Braune and Gaskin 1987; Furness et al. 1986). All netting, banding and sampling

activities were performed under the requisite institutional, state, provincial and federal permits.

Chemical analysis

To remove any externally deposited mercury, feathers were rinsed through three cycles of acetone and deionized water and allowed to dry. Blood and feather samples were analyzed by cold vapor atomic absorption spectroscopy for total Hg using a Milestone® DMA-80 (Shelton, CT, USA). Methods for this instrument have been validated for solid and liquid tissue matrices in US EPA Method 7473 (U.S. EPA 2007). Feathers were analyzed by fresh weight; each P1 was analyzed as an individual feather while breast feathers were analyzed as composites of four feathers from a single individual to account for intra-individual variation. Approximately 10–40 µl of blood was analyzed by wet weight for each individual. All values are reported here in ppm (µg g^{-1}) ± standard error (SE). Since MeHg has been found to represent ≥90% of the total Hg present in blood and feather tissues in other insectivorous passerines, we use total mercury from this analysis as a reflection of MeHg exposure.

The minimum detection limit for this analysis was 0.153–0.1688 ng. A method blank, matrix spike (blood only) and standard reference material (DOLT-4, dogfish liver, or DORM-3, fish protein, (National Research Council Canada)) were run every 12–20 samples for quality assurance. Recovery of total mercury for both standard reference materials ranged from 90 to 112%, with an average recovery of 100.86% ± 0.58 SE. Matrix spike recovery ranged from 99.4 to 115.7%, averaging 107.48% ± 0.99 SE.

Statistical analysis

Data were analyzed for statistical relationships using SAS version 9.1. Power analyses were performed for statistical tests using the SAS procedure, proc glmpower. Only samples with Hg levels higher than the method detection limit were included in analyses. Power levels were consistently above 0.85 for all analyses. Data in each test were assessed for normality using the Shapiro-Wilks Test as well as graphical representations of the data. Mercury data for all tissues met the assumptions for parametric statistical analysis after $\log_{10}$ transformation. A significance level was established at $P < 0.05$.

During the 2008–2009 winter season in North Carolina, five Nelson's Sparrows were captured multiple times. Feathers were not re-sampled from these individuals recaptured within the same season, but multiple blood samples were obtained. For this analysis, we include data from only the blood sample obtained at the first capture to ensure independence of data points and retain potential temporal relationships with feather samples from the same individual. North Carolina data were tested for differences in Hg among the three capture sites and across capture month (October 2008–April 2009). No significant differences in Hg concentrations were detected in any of the three sampled tissues across capture sites or months. Consequently, data were pooled across sites and months, and Nelson's Sparrows captured on North Carolina winter sites were treated as being from one location (NC). Nelson's Sparrows sampled from the two breeding sites were considered to be from independent locations (GF-ND and JB-ON).

During the winter, we were unable to determine the sex of captured individuals, but we assume that our samples represent a comparable number of males and females since capture methods should not have been biased for one over the other. However, the use of conspecific call playback methods during the breeding season resulted in the capture of nearly all males; only one female was captured with this method. Since data from Shriver et al. (2006) suggest that there is no significant difference in blood Hg levels between males and females of this species, we have included all of the data in our analysis.

The SAS procedure proc glm was used to test for differences in $\log_{10}$ transformed Hg data within each tissue type across locations. These models included the dependent variables breast feather Hg, first primary feather Hg and blood Hg and the independent variable location (NC, GF-ND and JB-ON). Tukey, Scheffe and Bonferroni post hoc tests were performed to detect pairwise differences between groups.

Results

We successfully captured and obtained blood and feather samples from wintering NSTS in North Carolina, breeding NSTS-N at GF-ND and breeding NSTS-A at JB-ON. We detected significant differences in breast feather mercury across sites ($F_{2,93} = 13.01$, $P < 0.0001$; Fig. 2a). Breast feathers from wintering NSTS contained an average mercury concentration of 2.94 ppm ± 0.37 SE, significantly higher than breeding NSTS-N (0.98 ppm ± 0.14 SE; $P < 0.0001$, pairwise comparison) and NSTS-A (1.21 ppm ± 0.36 SE; $P = 0.0017$, pairwise comparison). Of the three tissue types sampled, primary feathers held the highest average concentrations of mercury at each location, ranging from 3.27 ppm ± 0.63 SE in NSTS-A to 5.31 ppm ± 0.91 SE in NSTS-N (Fig. 2b). However, no significant differences in Hg were detected across locations for this tissue.

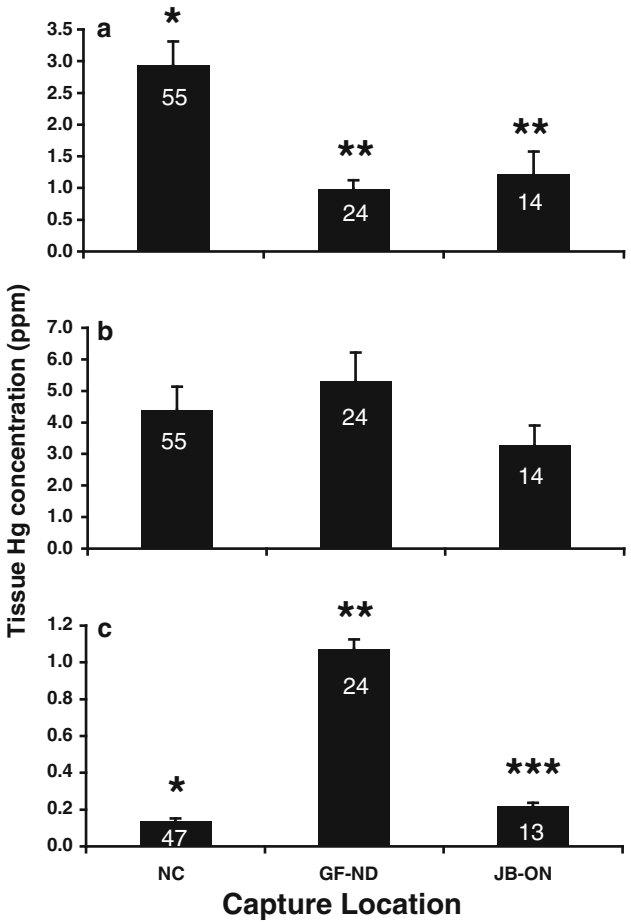

Fig. 2 Average Nelson's Sparrow tissue mercury (Hg) concentrations from wintering and breeding sites. **a** Breast feather Hg (fw), **b** First primary feather Hg (fw) and **c** Blood Hg (ww). All values are in ppm (μg g^{-1}); error bars represent standard error of the mean, and numbers within bars represent sample size. NC represents pooled data from winter captures at three sites near Wrightsville Beach, North Carolina, USA; GF-ND represents breeding captures near Grand Forks, North Dakota, USA; JB-ON represents breeding captures from the shore of James Bay North of Moosonee, Ontario, Canada. Stars denote significant differences among groups, ANOVA, $P < 0.05$

Blood Hg also varied across sites ($F_{2,84} = 264.88$, $P < 0.0001$) with highest levels found in NSTS-N with an average of 1.07 ppm ± 0.05 SE ($P < 0.0001$ for both sets of GF-ND pairwise comparisons). NSTS-A had significantly lower blood Hg (0.22 ppm ± 0.02 SE) than the breeding NSTS-N and significantly higher blood Hg than wintering NSTS (0.14 ppm ± 0.02 SE; $P < 0.0001$, pairwise comparison; Fig. 2c).

Discussion

Blood mercury

The regional patterns we detected for blood Hg concentrations did not match our predictions. Average NSTS-N blood Hg concentration (>1 ppm) was 7.6 times as high as average wintering NSTS blood Hg and 2.6 times as high as average blood Hg levels reported for breeding NSTS-S from salt marsh sites in ME (Shriver et al. 2006). NSTS-N blood Hg concentrations were also 1.6 times as high as those for Saltmarsh Sparrows (*Ammodramus caudacutus*) from Maine sites previously characterized by high bioavailability of Hg due to high coincident rates of Hg and acid deposition (Shriver et al. 2006). However, NSTS-N blood Hg concentrations were only about half as high as those from Massachusetts Saltmarsh Sparrow populations where habitat degradation for this species may be at its greatest (Lane et al. 2008). NSTS-N blood Hg is also approaching levels documented in Maine populations of the Common Loon (*Gavia immer*), a piscivore (1.73 ppm ± 0.06 SE; Evers et al. 2008). Our data fail to support our original hypothesis and indicate that higher regional levels of Hg deposition may not be the sole driving factor in determining Hg exposure in some ecosystems.

There are several possible explanations for the observed elevated blood Hg concentrations in breeding NSTS-N. One is simply that this subspecies is feeding at a higher trophic level than any of the other populations of Nelson's Sparrow for which blood Hg data exist. A recent study using stable isotopes in blood and feathers of wintering Nelson's Sparrows indicated that this species does not feed at a significantly different trophic level in the summer as compared to the winter (Michaelis 2009), making the first explanation an unlikely determinant of our results.

A second possible explanation is that the elevated blood Hg in NSTS-N represents point source pollution rather than the non-point source Hg deposition that is generally regarded as the primary factor in determining Hg exposure in a given area. Eastern Bluebirds (*Sialia sialis*) at a point source contaminated site in Virginia exhibited blood Hg levels of 1.21 ppm ± 0.57 SE (Condon and Cristol 2009). These data indicate that NSTS-N Hg intake near GF-ND is comparable to that of other songbirds at a point source contaminated site.

A third possibility is that the bioavailability of Hg in the GF-ND region is higher than that in the other ecosystems represented in this study. The bioavailability of Hg and other heavy metal contaminants is known to vary with physical properties of wetland and salt marsh ecosystems such as hydrology (Gambrell 1994; Williams et al. 1994), sediment characteristics (Williams et al. 1994; Gambrell 1994; Hung and Chmura 2006), and water and sediment pH (Williams et al. 1994; Gambrell 1994; Doka et al. 2003). Tree Swallow (*Tachycineta bicolor*) nestlings were sampled in northwestern North Dakota; Hg concentrations were higher in samples collected near seasonal wetlands compared to those near semi-permanent wetland or lakes

(Custer et al. 2008). This result indicates that there are inherent differences in mobility and availability of Hg contamination across these types of ecosystems. The sites at which we were able to capture NSTS-N were, for the most part, within seasonal wetland habitat and so could exhibit higher levels of Hg bioavailability for this reason.

Based upon our data and previous relevant research, we believe our results reveal unexpected regional differences in blood Hg that are most likely due to a combination of relatively elevated local Hg contamination and physical factors increasing Hg bioavailability at inland breeding sites. However, further study is certainly warranted in order to more fully understand these patterns since only a small number of studies has focused on understanding Hg contamination in any of these regions, and songbirds have, for the most part, been overlooked until recently with regards to mercury exposure.

Feather mercury

Our study presents the first robust documentation of feather Hg concentration in Nelson's Sparrows. Molt timing in this species has not been well-studied, but our results suggest that Nelson's Sparrows undergo a complete body molt twice a year confirming an earlier description of this process (Woolfenden 1956). The feathers sampled during the summer would have been grown in late winter before spring migration and would be representative of body burden at that point; feathers sampled in the winter would likewise retain a record of body burden prior to fall molt and migration. This hypothesis is corroborated by higher blood Hg levels, representing local dietary intake, for each population of breeding Nelson's Sparrow that has been sampled thus far compared to wintering populations in North Carolina. Our data indicate that, at the population level, Hg concentrations in breast feathers may be more closely linked to blood Hg concentrations than those in P1. Nelson's Sparrow breast feather Hg may represent an intermediate exposure duration between short-term exposure in blood and yearly accumulation in P1. Our results indicate that primary feathers may only be molted once per year and so would represent an entire year's worth of dietary Hg accumulation, integrating exposures from breeding and wintering sites.

The Hg concentrations observed in the three tissues sampled was surprising given the omnivorous habits of this species. The average NSTS-N feather Hg concentration exceeded 5 ppm, a minimal threshold above which negative reproductive effects may occur (Burger and Gochfeld 1997; Evers et al. 2004). However, all average Nelson's Sparrow P1 Hg concentrations were lower than those documented for Tree Swallows from a point source contaminated site in Virginia (Brasso and Cristol 2008) and Common Loons in Maine (Evers et al. 2008) which have both been documented to be experiencing decreased reproductive success attributed primarily to high levels of Hg exposure. Further study is necessary to determine whether Nelson's Sparrows' exposure to Hg has any effect on reproductive output and/or population declines across its range.

It is interesting to note that while blood Hg concentrations from NSTS-N are comparable to those from breeding NSTS-S and Saltmarsh Sparrows and from piscivorous Common Loons from relatively more degraded New England sites, feather Hg comparisons among these same species imply that NSTS-N may maintain the lowest yearly body burden accumulation of these four groups (Evers et al. 2008; Lane et al. 2008; Shriver et al. 2006). This result could indicate that the levels of Hg found in breeding NSTS-N blood may persist for only part of the summer. Members of the order Araneae (spiders) have been shown to contain Hg levels several times higher than those of other invertebrate prey items common to many songbirds (Cristol et al. 2008). Further study regarding the diet of Nelson's Sparrow is necessary to determine if spiders (or another prey item high in Hg) represent a significant but transient portion of their summer diet.

Conclusions

All three subspecies of Nelson's Sparrow have now been documented to exhibit higher blood Hg levels while residing on their respective breeding grounds compared to exposure at winter sites in North Carolina. We suggest that the non-breeding portion of the year, comprising more than half of a bird's lifetime, may be a critical period during which birds are exposed to lower levels of Hg contamination. For this reason, conservation efforts and ecological monitoring on winter sites should be considered just as essential to species protection efforts as those on breeding sites.

We are continuing to monitor Nelson's Sparrow Hg exposure on its previously unstudied inland breeding and coastal wintering sites. The non-lethal capture and sampling methods used in this study give us the potential to recapture individuals across seasons and to monitor possible Hg bioaccumulation in various tissue types over broad geographic regions. This approach allows us to address the question of how blood Hg dynamics is related to annual physiological and ecological changes. Data from this study indicate that knowledge of local Hg deposition rate is not enough to predict Hg exposure in some ecosystems.

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