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Mercury in non-breeding sparrows of North Carolina salt marshes

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Abstract We captured Nelson's, Saltmarsh and Seaside Sparrows (*Ammodramus nelsoni*, *A. caudacutus* and *A. maritimus*) at three salt marsh sites near Wrightsville Beach, North Carolina during five non-breeding seasons (September through April, 2006–2011). We analyzed breast feather samples from all of these seasons and blood and first primary feather (P1) samples from three seasons (2008–2011) for mercury (Hg). Generalized linear models were used to test for the impact of species, season, site and month on blood Hg, species, season and site on P1 Hg and species and season on breast feather Hg. The best-fit model for blood indicated that Hg varied among species, seasons and months. Saltmarsh Sparrows maintain higher blood Hg than Nelson's and Seaside Sparrows during the non-breeding season while they are feeding in mixed flocks. In Nelson's and Seaside Sparrows, blood Hg decreased during mid-winter compared to early fall and late spring. Breast feather and P1 Hg varied among species with Saltmarsh Sparrows exhibiting higher concentrations than the other two species, while Nelson's Sparrows had lower concentrations than the other two species. Breast feather Hg was higher in the final three seasons than in the first two. Our results indicate that Hg exposure on breeding sites may be increasing and that high levels of Hg exposure during the breeding season may affect blood Hg concentrations year-round in Saltmarsh Sparrows. Our data thus provide a baseline for future Hg assessments in these species in NC.

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Introduction

Mercury (Hg) is a global pollutant that has been found to negatively impact ecosystems and human populations (Clarkson and Magos 2006; Scheuhammer et al. 2007). In sediments, Hg can be converted by sulfur-reducing microbes to methylmercury (MeHg) which biomagnifies with increasing trophic position (Wolfe et al. 1998). Hg methylation in marsh habitats can occur at rates as much as 25 times as high as those in open water locations because of marsh hydrology, acid–base status and sediment characteristics (Marvin-DiPasquale et al. 2003; Williams et al. 1994). Factors that affect the accumulation and methylation of Hg in salt marsh and wetland habitats vary spatially (basin size, land use, soil properties, acid/base status, climate) and temporally (water discharge, water chemistry, redox conditions) (Williams et al. 1994), resulting in complex Hg dynamics and the potential for high levels of local variability in these processes.

Research on wild bird populations has demonstrated that Hg can be an environmental concern with regard to its effects on reproduction, behavior and survival (Brasso and Cristol 2008; Evers et al. 2008; Hallinger et al. 2011). In some bird species, for example, Hg levels of 5–40 ppm in feathers and 3.0 ppm in blood have been related to impaired reproduction and subsequent population declines (Brasso and Cristol 2008; Evers et al. 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 (Bechard et al. 2009). These conflicting results demonstrate our lack of adequate understanding of the species-specific causes of Hg toxicity.

Nelson's, Saltmarsh and Seaside Sparrows (*Ammodramus nelsoni*, *A. caudacutus*, and *A. maritimus*, respectively) co-occur in mixed flocks in North Carolina (NC) salt marshes during their non-breeding season. All three of these species inhabit salt marsh ecosystems during their non-breeding period, while Saltmarsh and Seaside Sparrows are salt marsh obligate species year-round (Fig. 1). In part because salt marshes represent some of the most functionally degraded habitats in North America (Greenlaw and Woolfenden 2007), each of these species is of conservation concern (Dettmers and Rosenberg 2000; IUCN 2010; Rich et al. 2004; USFWS 2002). Previous studies have characterized Hg exposure throughout portions of the ranges of Nelson's and Saltmarsh Sparrows, reporting higher than expected and geographically variable levels of Hg in the tissues of these omnivorous songbirds (Cristol et al. 2011; Lane et al. 2011; Shriver et al. 2006; Winder and Emslie 2011). For these reasons, and because of the environmental threats already identified for these species (DiQuinzio et al. 2001; Greenlaw and Woolfenden 2007), further research is necessary to determine when, where and how Hg levels may pose an additional threat.

Here, we use feathers and blood as non-destructive tools to examine Hg contamination in non-breeding sparrows. Feather Hg is widely accepted as an indicator of relatively long-term Hg exposure over a period of months or years, depending on molt patterns (Braune and Gaskin 1987; Evers et al. 2005). Feather Hg is typically comprised of ≥90% MeHg regardless of total Hg loads (Bond and Diamond 2009; Braune and Gaskin 1987) and reflects the amount of Hg in blood at the time of feather growth, which

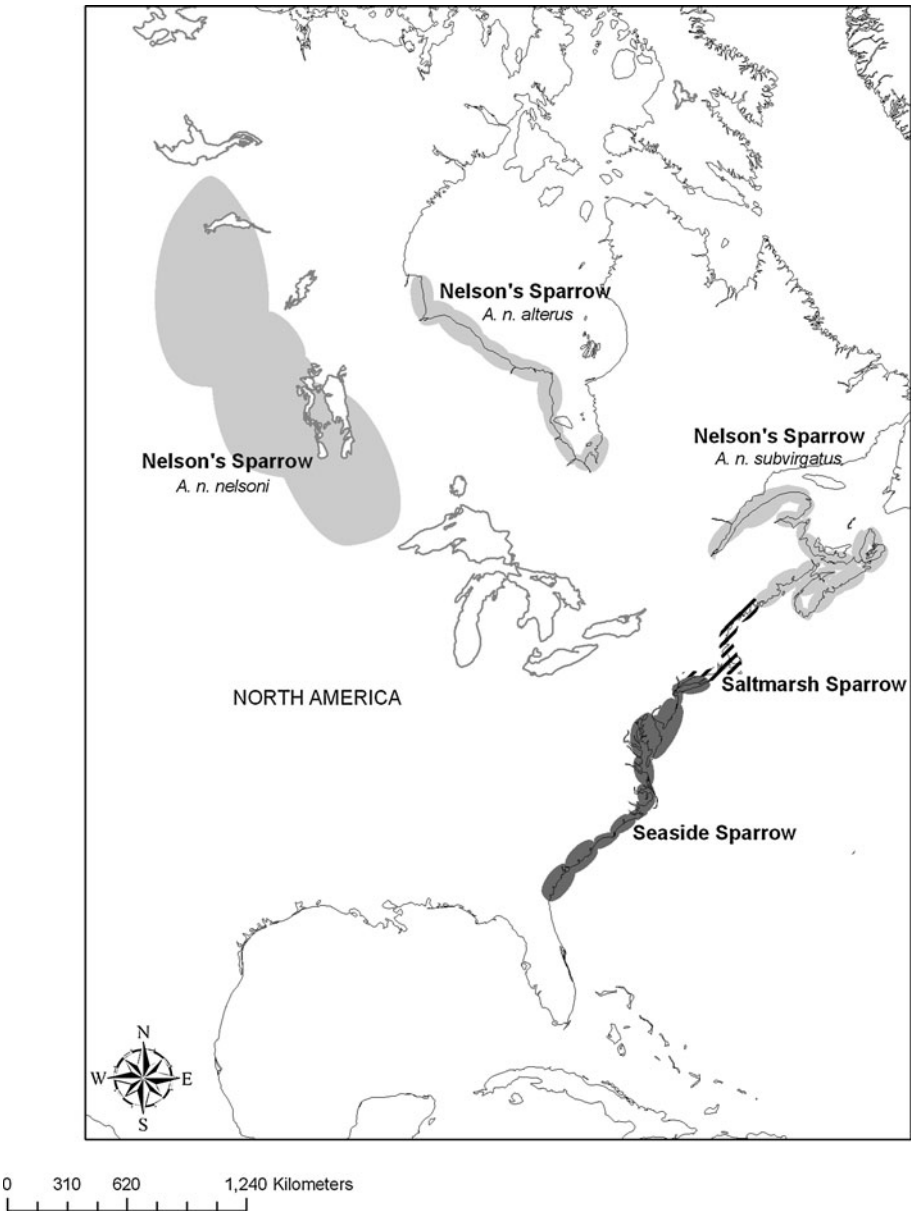

Fig. 1 Map of Nelson's (*dark gray*), Saltmarsh (*diagonal stripes*) and Seaside Sparrow (*dark gray*) global breeding ranges

is in turn influenced by overall body burden as muscle proteins (and accompanying Hg stores) are mobilized into blood for deposition in growing feathers (Bearhop et al. 2000a; Evers et al. 2005). Molt sequences are in need of further characterization in the coastal sparrow species in this study. However, based on the current available information for these species, breast feathers are molted biannually—once in a prebasic molt that usually takes place after breeding while still on breeding grounds prior to fall migration and again in a prealternate molt which usually takes place on non-breeding grounds prior to spring migration (Pyle 1997; F. Smith, unpubl. data). A molt of all nine primaries is thought to occur only once a year for each of these species, usually during a prebasic molt on breeding grounds prior to fall migration (Pyle 1997; F. Smith, unpubl. data). Based on this information, Hg in breast feathers sampled in the non-breeding season should be indicative of diet (and subsequent Hg body burden) during the breeding period, and P1 should be indicative of year-round Hg intake, integrating breeding and non-breeding Hg signals (Bearhop et al. 2000a; Evers et al. 2005).

Blood Hg has been suggested as the best evaluator of short-term dietary Hg uptake (Evers et al. 2005) and typically contains greater than 95% MeHg for both piscivorous (Evers et al. 2003; Fournier et al. 2002) and insectivorous bird species (Rimmer et al. 2005; Wada et al. 2009). Past work at our study sites has demonstrated that banded sparrows have maintained nearly complete fidelity to their original capture site (Michaelis 2009; Winder unpubl. data). Therefore, blood Hg values should reflect Hg availability on a highly localized scale.

Studies of wild birds on their non-breeding or winter habitats can provide baseline data to monitor future changes in these habitats, which are just as critical to their survival as the breeding habitats (Holmes 2007; Marra et al. 1998). An added advantage of conducting these studies at non-breeding habitats is that birds can be captured and sampled without disturbing nesting activities, and carefully selected tissues are representative of Hg exposure at both breeding and non-breeding sites (Bearhop et al. 2000a; Evers et al. 2005). We employ an information-theoretic approach to formally estimate the strength of evidence for various alternative hypotheses about the effect of species, site, season or month on tissue Hg concentrations.

Methods

Study sites and capture methods

All netting, banding and sampling activities were performed under the requisite institutional, state and federal permits. Nelson's, Saltmarsh and Seaside Sparrows were captured in mist nets on small, elevated islets in salt marshes during high tides and were banded with US Geological Survey aluminum bands. Birds were actively funneled into mist nets as we flushed them from one side of the islet toward one or two nets that were set perpendicular to the long axis of the islet. During the non-breeding season, all three subspecies of Nelson's Sparrow are present in mixed flocks on our study sites. Identification to subspecies was not always possible due to the considerable overlap in plumage and morphometric characteristics of these groups (Greenlaw and Rising 1994). Therefore, for the purposes of this study, all Nelson's Sparrow captures were pooled. We sampled at three islets near Wrightsville Beach, NC (Fig. 2) from September to April for five non-breeding seasons (2006–2011). These three islets are Lea-Hutaff (LH, near Lea-Hutaff Island), Parnell (P, first sampled by J. Parnell in the 1960s and 1970s), and Estuarine Reserve (ER, within the Masonboro Island National Estuarine Research Reserve). The LH site has been designated an Important Bird Area by Audubon NC, in part because of the presence of coastal sparrow species at this site.

Blood was sampled from the brachial vein with a sterile 26G1/2 needle; up to 70 µl was collected using a heparin-coated capillary tube. Capillary tubes were capped with Crito-caps® and stored in plastic vials to prevent breakage. Blood samples were initially stored in a cooler with ice; after returning to the laboratory, samples were stored at −80°C until analysis. The first primary feather (P1) was cut as close to the base of the shaft as possible, and 8–10 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 therefore provides the most consistent and relevant signal of body burden at the time of feather growth (Furness et al. 1986).

Hg analysis

To remove any externally deposited Hg, feathers were rinsed through three cycles of acetone and deionized water and allowed to dry. Breast feathers were collected throughout all five non-breeding seasons (2006–2011) but blood and P1 samples were collected only in the final three non-breeding seasons (2008–2011). All samples were analyzed for total mercury by thermal decomposition, catalytic conversion, gold-amalgamation and atomic absorption spectroscopy using a Milestone® DMA-80 (Shelton, CT, USA). Briefly, samples are dried and thermally decomposed; Hg is reduced to elemental its elemental state (Hg^0) and trapped with gold-amalgamation. When the amalgamator is heated, all of the trapped Hg is released to the atomic absorption spectrophotometer where its absorbance at 253.7 nm is representative of Hg content in the sample.

Fig. 2 North Carolina sampling sites. 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

These methods have been validated for solid and liquid tissue matrices in US EPA Method 7473 (U.S. EPA 2007). Feathers were analyzed by fresh weight (fw); 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 (Becker et al. 2002; Bond and Diamond 2008). Approximately 10–40 µl of blood was analyzed by wet weight (ww) for each individual. All values are reported here in ppm (µg g^{-1}) ± standard error (SE).

The minimum instrument detection limit during the period of sample analysis ranged from 0.153 to 0.1688 ng; only samples with Hg content above this limit were included in analyses. 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 Hg for 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. In the absence of adequate material for analysis of duplicate samples, matrix spikes served as a proxy for sample duplicates since recovery of Hg from both the standard reference material and sample matrix must be precise in order to achieve quality assurance results within acceptable limits.

Statistical analysis

To address multiple alternative hypotheses on the influence of species, season, month and site on tissue Hg, we used separate sets of generalized linear models (GLMs) for each tissue type in an information-theoretic approach. Each of these models included one or more of the variables we believed could reasonably contribute to variation in tissue Hg concentrations in coastal sparrows. With this approach, we identify the variables that exhibit the strongest evidence for affecting tissue Hg dynamics in these species.

The fully parameterized GLM for blood Hg was constructed as follows: Hg = species + season + month + site + species × season + species × month + species × site. The fully parameterized GLM for P1 Hg was: Hg = species + season + site + species × season + species × site. The fully parameterized GLM for breast feather Hg was: Hg = species + season + species × season. Month was not considered as an independent variable for either P1 or breast feather Hg since Hg deposited in feathers is biologically unavailable after the completion of feather growth, so no variation among months is expected (Burger and Gochfeld 1997). Likewise, site was not considered as an independent variable for breast feather Hg since these feathers were molted on breeding sites and should be representative of body burden influenced by dietary intake on those breeding sites rather than sites from the previous winter (Bearhop et al. 2000a; Pyle 1997). The remaining models for each tissue type were iterations of these full models having one or more independent variables and/or interaction terms removed. This resulted in a total of 34 candidate models for blood Hg, 12 for P1 Hg and 4 for breast feather Hg.

For each model set, competitors for well-supported models were identified using Akaike information criteria adjusted for small sample size (AIC_c; Akaike 1973; Anderson et al. 2001; Table 4 [Appendix]). Our model notation follows Anderson et al. (2001). Models with $\Delta AIC_c < 2.0$ that differed from the best-supported model by only one parameter (or with $\Delta AIC_c < 4.0$ that differed by two parameters, etc.) were excluded from consideration as competitive models on the basis of their inclusion of non-informative parameters (Arnold 2010; Burnham and Anderson 2002).

The relationships between tissue Hg and the factors from the best-fit model(s) for each tissue were then explored further using Tukey multiple-comparison post-hoc analyses (Anderson and Burnham 2002). Data were assessed for normality using the Shapiro-Wilks Test as well as graphical representations of the data. Tissue Hg data met the assumptions for parametric statistical analyses after $\log_{10}$ transformation. We present non-transformed values throughout with Hg concentrations expressed as arithmetic mean (ppm) ± standard error (SE) unless otherwise indicated. A significance level was established at $P < 0.05$. Data were analyzed using SAS version 9.1. When an individual was captured more than once, only data from the first capture were used in these analyses in order to maintain independence of data points.

Results

For each tissue type, a single model was identified from the set of competitors as the only model that carried appreciable support. In each case, no other model performed with a ΔAIC value < 2.0, or warranted substantial Akaike weight (Table 4 Appendix).

Blood Hg

The most parsimonious model for blood Hg was: Hg = species + season + month + species × month ($w_i = 0.996$; Table 1). Mean blood Hg concentrations ranged from 0.050 ± 0.005 ppm ww for Nelson's Sparrows in February 2011 to 0.361 ± 0.033 ppm ww for Saltmarsh Sparrows in November 2009 (Fig. 3). Post-hoc Tukey's multiple-comparison tests in exploration of the relationships between species, season and month to blood Hg revealed that blood Hg concentrations were significantly lower in Nelson's and Seaside Sparrows compared to Saltmarsh Sparrows ($P < 0.0001$ for both comparisons; Fig. 3). Regardless of species, blood Hg concentrations were significantly lower in the 2010–2011 sampling season compared to 2008–2009 and 2009–2010 ($P = 0.0002$ and 0.0006, respectively). Further exploration of the species × month interaction revealed differences among months within Nelson's and Seaside Sparrows. For these two species, blood Hg decreased during the middle of the non-breeding season with higher concentrations in early fall and late spring. Specifically, blood Hg was higher in October, November and December compared to February ($P \leq 0.02$) and higher in November compared to March ($P = 0.0023$) in Nelson's Sparrows. Similarly, blood Hg was higher in October, February, March and April compared to November and December ($P \leq 0.01$), and higher in October compared to January ($P = 0.0079$) in Seaside Sparrows. No differences were observed in blood Hg among months for Saltmarsh Sparrows.

Feather Hg

The most parsimonious model for P1 Hg was: Hg = species + site ($w_i = 0.983$; Table 1). Mean P1 Hg concentrations ranged from 2.796 ± 0.346 ppm fw for Nelson's Sparrows at ER to 16.969 ± 2.001 ppm fw for Saltmarsh Sparrows at LH (Table 2). Post-hoc Tukey's multiple comparisons tests indicated significant differences between each pair of species ($P < 0.0001$ for all comparisons;

Table 1 Best-supported models and accompanying generalized linear model statistics for tissue Hg concentrations

Model	Source	df	*F* value	*P* value	*R* Square
Blood Hg = species + season + site + species × month	Overall model	23	14.81	<0.0001	0.358
	Species	2	19.04	<0.0001	
	Season	2	11.61	<0.0001	
	Month	7	7.62	<0.0001	
	Species × month	12	10.84	<0.0001	
First primary feather Hg = species + site	Overall model	4	33.18	<0.0001	0.161
	Species	2	51.42	<0.0001	
	Site	2	10.73	<0.0001	
Breast feather Hg = species + season	Overall model	6	41.16	<0.0001	0.217
	Species	2	89.74	<0.0001	
	Season	4	19.05	<0.0001	

Models were selected using AIC_c from candidate model sets (Table 4 Appendix). Blood and feathers were sampled from Saltmarsh, Seaside and Nelson's Sparrows (*A. caudacutus*, *A. maritimus*, and *A. nelsoni*) during their non-breeding period (September through April) in NC salt marshes from 2006 to 2011

Table 2) with Saltmarsh Sparrows exhibiting highest and Nelson's Sparrows exhibiting lowest Hg concentrations. P1 Hg was also influenced by site. Regardless of species, P1 Hg concentrations were lower at ER than either LH or P ($P < 0.0001$ and 0.0014, respectively).

The most parsimonious model for breast feather Hg was: Hg = species + season ($w_i = 1.000$; Table 1). Mean breast feather Hg concentrations ranged from 1.816 ± 0.334 ppm fw for Nelson's Sparrows in the 2006–2007 season to 8.925 ± 0.996 ppm fw for Saltmarsh Sparrows in the 2010–2011 season (Table 3). Post-hoc Tukey's multiple comparisons tests indicated that breast feather Hg concentrations followed the same pattern among species as described above for P1 Hg ($P < 0.0001$ for all pairwise comparisons; Table 3). Breast feather Hg varied among sampling seasons with significantly lower concentrations in 2006–2007 and 2007–2008 than in the final three seasons ($P \leq 0.009$ for all pairwise comparisons; Table 3).

Discussion

For each tissue type, our statistical approach identified a single model, representing a specific alternate hypothesis, which received the vast majority of support in explaining variation in our data. From those well-supported models, we have detected factors that contribute to tissue Hg levels in Nelson's, Seaside and Saltmarsh Sparrows, and careful consideration of the time frame of Hg exposure represented in each tissue has allowed us to form hypotheses on how these factors may be interrelated in overall Hg dynamics. The dependence of Hg availability on temporally and spatially dynamic and interrelated factors leads to the potential for Hg to vary across space and time (Edmonds et al. 2010; Lane et al. 2011; Lane and Evers 2007). Therefore, it was not unexpected that parameters representing time and/or space should receive strong support in the most parsimonious models for each tissues we examined.

We observed a decrease in blood Hg for Nelson's and Seaside Sparrows between fall arrival and mid-winter, followed by a subsequent increase between mid-winter and late spring (Fig. 3). One explanation for this pattern is a diet shift toward higher proportions of plant material during mid-winter as invertebrate prey become scarce. Since Hg exposure is generally higher on breeding sites compared to non-breeding sites for these species (Cristol et al. 2011; Lane et al. 2011; Shriver et al. 2006; Winder and Emslie 2011), a decrease in Hg consumption related to geography (regardless of trophic position) could complement a seasonal trophic shift as an explanation for the observed decrease in blood Hg within the non-breeding period.

Recent restrictions on Hg emissions in North America have limited Hg deposition, but global cycling of previous pollution continues, and some areas continue to experience an increase in Hg loading (Braune et al. 2005; Frederick et al. 2004). Since breast feathers sampled in the non-breeding season should be indicative of exposure during the breeding season (Bearhop et al. 2000a; Evers et al. 2005; Pyle 1997), the increase in breast feather Hg that we observed over time may indicate that Hg availability and/or contamination increased over this time period in the salt and freshwater marshes that serve as breeding habitat for these sparrows. Conversely, we observed an overall decrease in blood Hg in 2010–2011 compared to 2008–2010, which may represent a decrease in local availability of Hg in southeastern NC salt marshes. Additional study is required to determine if the differences in blood and breast feather Hg we observed among seasons are part of larger trends or only represent stochasticity among seasons.

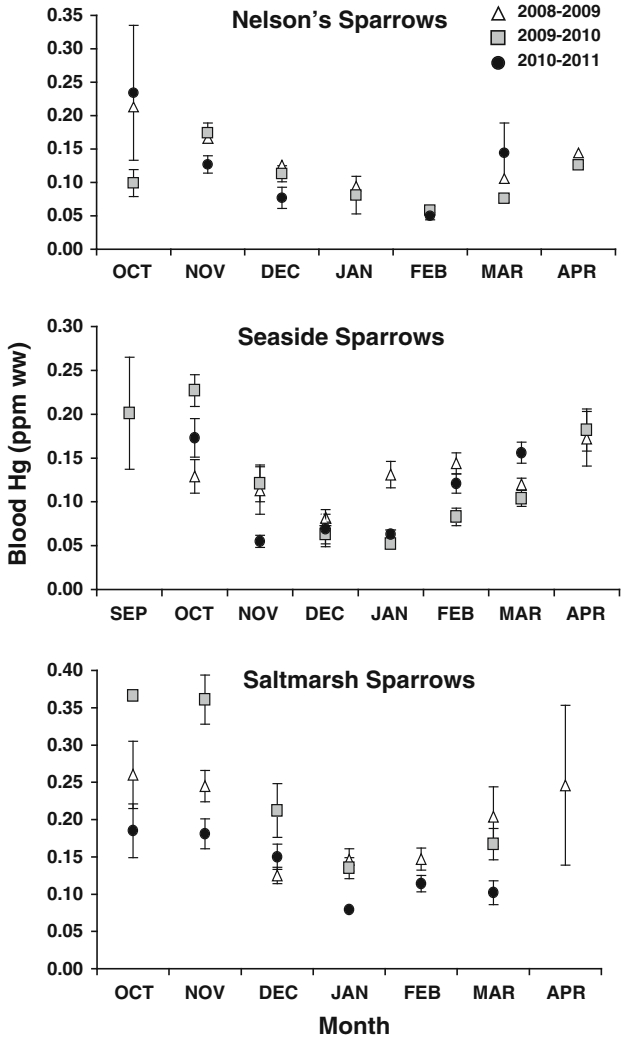

Fig. 3 Mean blood mercury (Hg) concentrations (ppm wet weight (ww)) for non-breeding sparrows sampled in North Carolina salt marshes from September through April (2008–2011). For all species, *open triangles* represent the 2008–2009 non-breeding season, *gray squares* represent 2009–2010, and *black circles* represent 2010–2011. *Error bars* represent standard error of the mean

Our results confirmed what other studies have already documented—that species differences are an important factor in explaining differences in tissue Hg in this group of sparrows (Cristol et al. 2011; Shriver et al. 2006; Winder and Emslie 2011). Relating these differences in Hg levels to other research on feeding ecology has allowed us to develop hypotheses regarding Hg dynamics in these species. Here we focus on what is known about dietary signals (δ^{15}N and δ^{13}C) and Hg levels in blood for these species since these should be indicative of recent dietary intake on shared non-breeding sites (Evers et al. 2005; Podlesak et al. 2005).

Hg has been reported to increase with increasing δ^{15}N (a proxy for trophic position) because of the capacity of Hg to biomagnify within a food web (Bearhop et al. 2000b). A study of Saltmarsh and Nelson's Sparrows at shared non-breeding sites in Virginia reported no difference between blood δ^{15}N signatures for these two species despite significant differences between blood Hg levels (Cristol et al. 2011). These authors also reported an enriched δ^{13}C signature in Saltmarsh compared to Nelson's Sparrows which was used as the basis for their hypothesis that these two species may forage within different food webs (with different relative inputs of C3 (*Juncus*) versus C4 (*Spartina*) photosynthesis) and for that reason, experience different levels of Hg exposure while feeding at similar trophic positions (Cristol et al. 2011).

Blood δ^{15}N data from previous research on the same three NC sites used in the present study indicate that Nelson's, Seaside and Saltmarsh Sparrows feed at similar trophic positions while blood δ^{13}C data indicate that Saltmarsh and Seaside Sparrows feed (to some extent) within a different food web than Nelson's Sparrows (Michaelis 2009). On average, Saltmarsh Sparrows blood Hg concentrations are 1.8 times as high as those of Seaside and Nelson's Sparrows in this study. As presented in Cristol et al. (2011), we believe that the disparity between blood Hg concentrations in Saltmarsh and Nelson's Sparrows could be based on their participation in different salt marsh food webs. However, we have no evidence that Saltmarsh and Seaside Sparrows are feeding at different trophic positions or within different food webs at our NC sites. Therefore, the difference between their blood Hg levels represents an apparent disconnect between dietary signals and Hg within these populations.

Table 2 First primary feather mercury (Hg) concentrations (ppm fresh weight (fw)) for wintering coastal sparrows captured from September through April in three non-breeding seasons (2008–2011)

Sparrows were captured at three sites in southeastern North Carolina salt marshes (Fig. 2)

Species	Site	N	Mean Hg (ppm fw) ± SE (Min.–Max.)
Nelson's Sparrows	ER	85	2.796 ± 0.346 (0.220–14.981)
	LH	100	4.023 ± 0.501 (0.192–31.500)
	P	74	5.343 ± 0.860 (0.398–38.724)
Seaside Sparrows	ER	121	5.401 ± 0.666 (0.288–46.754)
	LH	111	8.687 ± 0.834 (0.378–47.080)
	P	75	7.108 ± 0.758 (0.463–31.190)
Saltmarsh Sparrows	ER	22	9.300 ± 1.807 (1.151–30.889)
	LH	57	16.969 ± 2.001 (0.783–60.620)
	P	54	12.399 ± 2.005 (0.388–69.275)

Table 3 Breast feather mercury (Hg) concentrations (ppm fresh weight (fw)) for wintering coastal sparrows captured from September through April in five non-breeding seasons (2006–2011)

Species	Season	N	Mean Hg (ppm fw) ± SE (Min.–Max.)
Nelson's Sparrows	2006–2007	36	1.816 ± 0.334 (0.142–12.003)
	2007–2008	42	1.860 ± 0.202 (0.218–7.477)
	2008–2009	47	3.186 ± 0.420 (0.300–12.226)
	2009–2010	145	3.636 ± 0.340 (0.475–27.266)
	2010–2011	66	3.437 ± 0.309 (0.682–15.366)
Seaside Sparrows	2006–2007	26	3.236 ± 0.514 (0.567–12.644)
	2007–2008	36	2.571 ± 0.345 (0.682–9.479)
	2008–2009	94	4.081 ± 0.283 (0.688–14.617)
	2009–2010	117	4.168 ± 0.258 (0.592–21.260)
	2010–2011	97	4.700 ± 0.282 (0.867–13.687)
Saltmarsh Sparrows	2006–2007	21	6.260 ± 0.820 (0.973–14.887)
	2007–2008	36	4.522 ± 0.456 (0.527–13.654)
	2008–2009	57	5.607 ± 0.424 (1.138–13.630)
	2009–2010	36	6.412 ± 0.571 (0.464–13.545)
	2010–2011	40	8.925 ± 0.996 (0.747–24.855)

Sparrows were captured at three sites in southeastern North Carolina salt marshes (Fig. 2)

One explanation for this disconnect is that high levels of exposure to Hg on breeding sites could result in Saltmarsh Sparrows accumulating a higher body burden of Hg than they are able to excrete during feather growth. If this explanation were correct, it would indicate that Hg exposure during one part of a life cycle might continue to affect tissue levels long after that exposure has ended. Geographic differences in Hg exposure for Saltmarsh Sparrows are now well-documented with blood Hg concentrations on breeding sites ranging from 3 to 10 times as high as those on non-breeding sites (Cristol et al. 2011). A second possible explanation for the observed disconnect may be that Saltmarsh Sparrows are feeding on specific prey items that contain higher levels of Hg than prey consumed by Seaside Sparrows (though at a similar trophic position). This explanation is not parsimonious, but similar patterns have been documented in other ecosystems and have been attributed to spatial variation in MeHg production rates (Nisbet et al. 2002). We cannot discount either of these explanations, and further research is necessary to better understand species-specific Hg dynamics.

Feather Hg concentrations between 5 and 40 ppm fw have been linked to impaired reproduction and population instability in some species (Burger and Gochfeld 1997; Evers et al. 2008). While this range cannot serve as a definite threshold of negative effects, it can be viewed as a point at which concern and further investigation are warranted. In our study, breast feather Hg concentrations exceeded 5 ppm in 16% of Nelson's Sparrows, 25% of Seaside Sparrows and 55% of Saltmarsh Sparrows whose mean breast feather Hg concentration also exceeded this criteria (6.325 ± 0.312 ppm fw). For P1 Hg, 24% of Nelson's Sparrows, 50% of Seaside Sparrows and 58% of Saltmarsh Sparrows exceeded 5 ppm while 1% of Seaside Sparrows and 5% of Saltmarsh Sparrows exceeded 40 ppm. Mean P1 Hg exceeded 5 ppm for both Saltmarsh and Seaside Sparrows, (13.845 ± 1.238 and 7.006 ± 0.447 ppm fw, respectively). While these relatively high concentrations were unexpected, we have no evidence thus far that these levels of Hg exposure have detrimental effect on these species.

We have found that species, season and month contribute substantially to blood Hg concentrations, species and site contribute to P1 Hg, and species and season contribute to breast feather Hg. Though the top model for each tissue type was well-supported, those models explained only a modest proportion of variability in tissue Hg with r^2 values ranging from 0.16 to 0.36 (Table 1), thus other factors important to tissue Hg variability remain unidentified. Individual physiology is an example of an understudied component of Hg dynamics that may well outweigh any of the other factors we have considered in determining tissue Hg levels; this possibility deserves further attention.

Our approach of sampling both blood and feathers from individual birds has increased our understanding of Hg exposure in these species of conservation concern. Information-theoretic analyses have provided strength of evidence for specific factors that are influential to blood and feather Hg and so can be understood to be of importance during different parts of these sparrows' annual cycles. We know of no other study that has documented intra-annual variation in blood Hg concentrations in a wild songbird population. Our results provide insight on what may be driving higher Hg levels in Saltmarsh Sparrows compared to the other two species in our study during their non-breeding period. Based on conservative negative effects threshold values for feather Hg, 58% of the Saltmarsh Sparrows, 50% of Seaside Sparrows and 24% of Nelson's Sparrows we have sampled may be at risk because of Hg

exposure. Non-breeding sites in southeastern NC provide a period of lower Hg exposure during which sparrows may depurate Hg consumed on breeding sites. We believe these non-breeding sites should be considered vital to the conservation of these species and view our data as important baseline information for future assessment of Hg exposure in these species.

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Appendix

See Table 4.

Table 4 Candidate sets of competing generalized linear models compared using Akaike information criteria for small sample sizes (AIC_c)

Model	AIC_c	ΔAIC_c	w_i	k
Blood Hg = species + season + month + species × month	**149.0**	**0.0**	**0.996**	**39**
Species + month + species × month	160.9	11.9	0.003	36
Species + month + site + species × month	162.9	13.9	0.001	39
Species + month + site + species × month + species × site	170.1	21.1	0.000	48
Species + season + month + site + species × site	220.6	71.6	0.000	27
Species + season + month + site + species × season + species × site	223.7	74.7	0.000	36
Species + month + site + species × site	227.8	78.8	0.000	24
Species + season + month + site	233.3	84.3	0.000	18
Species + season + month	234.4	85.4	0.000	15
Species + season + month + site + species × season	234.9	85.9	0.000	27
Species + season + month + species × season	236.2	87.2	0.000	24
Species + month + site	242.4	93.4	0.000	15
Species + month	242.6	93.6	0.000	12
Species + season + site + species × site	252.5	103.5	0.000	19
Species + site + species × site	256.0	107.0	0.000	16
Species + season + site + species × season + species × site	258.2	109.2	0.000	28
Species + season	275.2	126.2	0.000	7
Species + season + site	275.8	126.8	0.000	10
Species + season + site + species × season	280.0	131.0	0.000	19
Species + season + species × season	280.2	131.2	0.000	16
Species + site	282.1	133.1	0.000	7
Species	283.7	134.7	0.000	4
Season + month + site	299.0	150.0	0.000	15
Season + month	309.4	160.4	0.000	12
Month + site	310.2	161.2	0.000	12
Month	320.3	171.3	0.000	9
Season + site	325.5	176.5	0.000	7
Site	331.3	182.3	0.000	4
Season	333.3	184.3	0.000	4
First primary feather Hg = species + site	**981.1**	**0.0**	**0.983**	**7**
Species + season + site	989.5	8.4	0.015	10
Species	993.2	12.1	0.002	4
Species + season	1002.1	21.0	0.000	7
Species + season + species × season	1007.8	26.7	0.000	16
Site	1068.4	87.3	0.000	4

Table 4 continued

Model	AIC_c	ΔAIC_c	w_i	k
Season + site	1068.9	87.8	0.000	7
Season	1088.7	107.6	0.000	4
Breast feather Hg = species + season	**446.4**	**0.0**	**1.000**	**9**
Species	499.1	52.7	0.000	4
Season	599.0	152.6	0.000	6

Models are designed to test multiple alternative hypotheses on the influence of species, season site or month on tissue mercury (Hg) concentrations. Different sets of parameters were chosen for each tissue type to address hypotheses specific and appropriate to that tissue type. Models with non-informative parameters were removed from consideration as candidate models and are not shown here (Burnham and Anderson 2002); however, a careful reading of our methodology should provide information on the structure of those models. Model notation follows Anderson et al. (2001): ΔAIC_c (simple difference between the AIC_c score for a particular model and that of the best-supported model; w_i (Akaike model weight); k (number of model parameters). For each tissue, the best-fit model is shown in bold

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