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Understanding associations between nitrogen and carbon isotopes and mercury in three *Ammodramus* sparrows

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ABSTRACT

We analyzed nitrogen and carbon stable isotope ratios and mercury (Hg) in breast feathers from three species of closely related sparrows, Saltmarsh, Seaside, and Nelson's Sparrows (*Ammodramus caudacutus*, *A. maritimus*, and *A. nelsoni*, respectively), to assess if trophic position and food web structure influence Hg exposure in these species. Sparrows were captured during the non-breeding season from 2006 to 2008 in North Carolina salt marshes near Wrightsville Beach, New Hanover County. Generalized linear models were used to test for the influence of species, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ on breast feather Hg. The most parsimonious model included species, $\delta^{15}\text{N}$, and their interaction term and explained 36% of the variation in breast feather Hg. Each species exhibited a different association between breast feather $\delta^{15}\text{N}$ and Hg with Seaside Sparrows showing a positive correlation ($r = 0.27$, $P = 0.03$), Nelson's Sparrows a negative correlation ($r = -0.28$, $P = 0.01$), and Saltmarsh Sparrows with no significant association. For Saltmarsh Sparrows, $\delta^{15}\text{N}$ and Hg revealed decoupling between breast feather Hg and trophic position. Our results demonstrate that the influence of $\delta^{15}\text{N}$ on breast feather Hg is likely indicative of geographic variation in $\delta^{15}\text{N}$ baselines rather than trophic position.

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1. Introduction

Mercury (Hg) biomagnifies [as methylmercury (MeHg)] in both aquatic and terrestrial food webs (Cristol et al., 2008; Evers et al., 2005) and can reach levels that result in negative effects on wildlife as well as human populations (Clarkson and Magos, 2006). Research on free-living birds has found that environmental levels of Hg can be related to decreased reproduction and survival as well as aberrant behavior (Brasso and Cristol, 2008; Evers et al., 2008; Hallinger et al., 2011; Jackson et al., 2011). In some bird species, Hg levels of 2.4–40 ppm in feathers or 0.7–3.0 ppm in blood have been related to impaired reproduction and subsequent population declines (Brasso and Cristol, 2008; Evers et al., 2008; Jackson et al., 2011). However, other species appear to behave and reproduce normally even with feather and blood Hg levels at the high end of, or even 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.

Stable isotope analysis (SIA) can complement the study of a biomagnifying contaminant and address questions on changes in contaminant exposure with shifting trophic position or food web structure (Thompson et al., 1998; Vo et al., 2011). While a substantial body of literature has examined the association between

feeding ecology and Hg exposure in seabirds (Anderson et al., 2009; Becker et al., 2002; Vo et al., 2011), only one study that we are aware of has attempted this with songbirds (Cristol et al., 2011). Therefore, our understanding of Hg variability among songbird populations with respect to dietary factors remains restricted.

Stable carbon and nitrogen isotopes have been shown to vary in a predictable fashion based on diet and consumer physiology (Atwell et al., 1998). The heavy or enriched form of nitrogen (^{15}N) increases from 3 to 5‰ with each increase in trophic level, while ^{13}C increases less dramatically at approximately 1‰ with each increase in trophic level (Fry et al., 1984; Minagawa and Wada, 1984). ^{13}C also varies with the pathway of carbon fixation in plants with C3 plants maintaining lower ^{13}C values than C4 plants (Hobson and Clark, 1992); in this way, ^{13}C signatures can be used to infer habitat type and food web structure (Marra et al., 1998). As with Hg, isotopic signatures are incorporated over the period of tissue formation, resulting in different tissues reflecting varying temporal scales of dietary information (Bearhop et al., 2002; Hobson and Clark, 1992).

Previous studies have examined the effects of trophic position on Hg concentrations in bird tissues and report contradictory results. Weak associations between $\delta^{15}\text{N}$ and Hg have led investigators to conclude that varying contaminant levels across foraging sites or food webs and individual physiology are more important than trophic position in determining consumer tissue Hg levels (Bearhop et al., 2000a; Cristol et al., 2011). In addition, Hg and carbon and nitrogen isotopes may represent diet over different time scales in the same tissue, resulting in a decoupling of the information from these two sources (Anderson et al., 2009; Bearhop et al., 2000a). Conversely, a

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strong positive association between $\delta^{15}\text{N}$ and Hg supports the hypotheses that Hg variation in consumer tissues is strongly influenced by trophic position and that Hg (in those systems) biomagnifies predictably with increasing trophic position (Atwell et al., 1998; Bearhop et al., 2000c; Vo et al., 2011).

Here, we use feather Hg and SIA data from Saltmarsh, Seaside, and Nelson's Sparrows (*Ammodramus caudacutus*, *A. maritimus* and *A. nelsoni*, respectively) to address hypotheses on how trophic position and food web structure influence Hg concentrations in these three closely related songbird species. Each of these species inhabits salt marsh ecosystems during its non-breeding period, while Saltmarsh and Seaside Sparrows are salt marsh obligate species year-round (Fig. 1 in Winder and Emslie, 2011b). 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, 2011; Rich et al., 2004; USFWS, 2002). Previous studies have characterized Hg exposure throughout portions of the ranges of these species, reporting higher than expected and geographically variable levels of Hg in the tissues of these omnivores (Cristol et al., 2011; Lane et al., 2011; Shriver et al., 2006; Winder and Emslie, 2011a; Winder and Emslie, 2011b). For these reasons, and because of the environmental threats already identified for these species (Bayard and Elphick, 2011; DiQuinzio et al., 2001; Greenlaw and Woolfenden, 2007; Lane et al., 2011), further research is necessary to determine when, where, and how Hg levels may pose an additional threat.

Our study is an exploration of Hg ecotoxicology in songbirds. We employed an information-theoretic approach to formally estimate the strength of evidence for various alternative hypotheses about the influence of species, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ on Hg concentrations in sparrow breast feathers. Our results, integrating toxicology and feeding ecology, expand our ability to establish the ecological relevance of changes in trophic interactions or Hg availability patterns across the ranges of these species.

2. Materials and methods

2.1. Study sites, capture and sampling methods

Nelson's, Saltmarsh, and Seaside Sparrows were captured in mist nets on small, elevated islets in salt marshes during high tide and were banded with USGS aluminum bands. All netting, banding, and sampling activities were performed under the requisite institutional, state and federal permits. Birds were actively funneled into mist nets as we flushed them from one side of the islet toward the net(s) 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 of these individuals 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, Nelson's Sparrow captures were not separated to subspecies.

We sampled at three islets near Wrightsville Beach, NC (Fig. 2 from Winder and Emslie, 2011b) from October to April for two non-breeding seasons (2006–2008). Hereafter, we use year to refer to a single non-breeding winter period spanning two calendar years. The three islet sites include 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 sparrow species at this site.

A sample of 8–10 breast feathers was plucked from each bird and stored in plastic re-sealable bags. For our three study 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). 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 reflects the amount of Hg in blood at the time of feather growth, which 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., 2000b; Evers et al., 2005). Thus, Hg in breast feathers sampled during the non-breeding season is indicative of Hg accumulated since the previous molt (here, chiefly representing breeding season diet; Bearhop et al., 2000b; Evers et al., 2005).

2.2. Hg analysis

To remove any externally deposited Hg, feathers were rinsed through 3 cycles of acetone and deionized water and allowed to dry. 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 its elemental state (Hg^0) and trapped with gold-amalgamation. When the amalgamator is heated, trapped Hg is released to the atomic absorption spectrophotometer where its absorbance at 253.7 nm is representative of Hg content in the sample (U.S. EPA, 2007). Feathers have been documented to contain $\geq 90\%$ MeHg regardless of total Hg loads (Bond and Diamond, 2009), so our analysis of total Hg should be analogous to MeHg levels. Breast feathers were analyzed by fresh weight (fw) as composites of four feathers from a single individual to account for intra-individual variation (Becker et al., 2002; Bond and Diamond, 2008). All values are reported here in ppm ($\mu\text{g g}^{-1}$) \pm standard error (SE).

The minimum instrument detection limit during the period of sample analysis ranged from 0.15 to 0.17 ng; only samples with Hg levels above this limit were included in analyses. A method blank, matrix spike (using blood samples from a separate study) 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 $101 \pm 1\%$ SE. Matrix spike recovery ranged from 99 to 116%, averaging $108 \pm 1\%$ 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.

2.3. Stable isotope analysis

Feathers were rinsed in distilled water and acetone to remove contaminants and left to dry for 24 h (Nisbet et al., 2002). Dry feathers were cut into small fragments, weighed, and placed in tin cups for analysis in the mass spectrometer. SIA was conducted using a Thermo DELTA V Plus (IRMS) interfaced with a Costech 4050 Elemental Analyzer located at the Center for Marine Science, University of North Carolina Wilmington. Raw δ values were normalized on a two-point scale using depleted and enriched glutamic acid standard reference materials USGS-40 ($\delta^{13}\text{C}$: -26.389 ± 0.042 ; $\delta^{15}\text{N}$: -4.5 ± 0.1) and USGS-41 ($\delta^{13}\text{C}$: 37.626 ± 0.049 ; $\delta^{15}\text{N}$: 47.6 ± 0.2). Sample precision based on duplicate standard reference materials was 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Stable isotope ratios are expressed in δ notation in per mill units (‰), according to the following equation:

$$\delta X = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where X is $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. The R_{standard} values were based on the V-PeeDee Belemnite (VPDB) for ^{13}C and atmospheric N_2 for ^{15}N .

2.4. Statistical analysis

Data were analyzed for statistical relationships using SAS version 9.1. We assessed isotope ratios and tissue Hg levels for differences among years using separate mixed models (PROC MIXED), each with breast feather Hg, $\delta^{15}\text{N}$, or $\delta^{13}\text{C}$ as a dependent variable and year as a random factor. None of these models indicated significant differences among years ($P > 0.05$), so data were pooled across years. To address multiple alternative hypotheses on the influence of species, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ on breast feather Hg, we used a set of generalized linear models (GLMs) in an information-theoretic approach. Each model included one or more of the variables we believed could reasonably contribute to variation in breast feather Hg concentrations.

The fully-parameterized GLM was constructed as follows: breast feather $\text{Hg} = \text{species} + \delta^{15}\text{N} + \delta^{13}\text{C} + \text{species} \times \delta^{15}\text{N} + \text{species} \times \delta^{13}\text{C} + \delta^{13}\text{C} \times \delta^{15}\text{N}$. The remaining GLMs in the candidate model set were iterations of this full model having one or more independent variables and/or interaction terms removed (Table 1). We used Akaike Information Criteria adjusted for small sample size (AIC_c ; Akaike, 1973; Anderson et al., 2001) to select well-supported models from our candidate model set. We use the term well-supported to refer to models that receive substantial Akaike weight (w_i) and so warrant selection as models that provide the best fit to the data given their number of parameters (Burnham and Anderson, 2002). Our model notation follows Anderson et al. (2001). Models with $\Delta\text{AIC}_c < 2.0$ that differed from the best-supported model by only one parameter (or with $\Delta\text{AIC}_c < 4.0$ 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 associations between breast feather Hg and the factors from well-supported models were explored further (Anderson and Burnham, 2002) using post-hoc simple Pearson correlations (PROC CORR), quantile regression (PROC QUANTREG) and Tukey multiple comparison tests (PROC GLM).

Data for all tests were assessed for normality using the Shapiro–Wilks Test as well as graphical representations of the data. Hg and isotope data met the assumptions for parametric statistical analysis after \log_{10} transformation; we present non-transformed values

throughout. A significance level was established at $P < 0.05$ for all tests. Hg values and isotope signatures are expressed as arithmetic means \pm SE unless otherwise indicated. 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.

3. Results

Our information-theoretic approach yielded only one model with $\Delta\text{AIC}_c < 2.0$ ($\text{Hg} = \text{Species} + \delta^{15}\text{N} + \text{Species} \times \delta^{15}\text{N}$; $w_i = 0.54$); we present this as the most parsimonious and best-fit model. However, three additional models warranted some support ($\Delta\text{AIC}_c < 3.0$; w_i ranging from 0.17 to 0.14; Table 1). Therefore, we conservatively present these four models as well-supported and use them to interpret factors that contribute to breast feather Hg (Table 2). Collectively, these models account for 99% of the support within our candidate model set (Table 1). Each well-supported model explained a moderate portion of the variation observed in breast feather Hg ($r^2 = 0.35\text{--}0.36$; Table 2).

We examined our candidate model set to determine the relative importance of variables (Arnold, 2010) and found that species, $\delta^{15}\text{N}$, the interaction term including these two main effects, and $\delta^{13}\text{C}$ received the most support of all of the parameters within our candidate models (Table 3; $\sum w_i = 1.00\text{--}0.46$). Using post-hoc analyses, we found that Saltmarsh Sparrows exhibited significantly higher breast feather Hg levels than Seaside Sparrows, which in turn exhibited higher levels than Nelson's Sparrows (Table 4). $\delta^{15}\text{N}$ also varied among species with Saltmarsh and Seaside Sparrows exhibiting more enriched signatures compared to Nelson's Sparrows (Table 4). The association between $\delta^{15}\text{N}$ and Hg varied among species (represented in the interaction term $\text{Species} \times \delta^{15}\text{N}$; $\sum w_i = 0.68$) with no significant association in Saltmarsh Sparrows ($P = 0.95$, $r = -0.01$, $n = 58$), a positive association in Seaside Sparrows ($P = 0.03$, $r = 0.27$, $n = 69$) and a negative association in Nelson's Sparrows ($P = 0.01$, $r = -0.28$, $n = 88$) (Fig. 1). No significant associations were detected between $\delta^{13}\text{C}$ and Hg for any of our three study species (Fig. 2); however, $\delta^{13}\text{C}$ varied among species with Saltmarsh and Seaside Sparrows exhibiting signals indicative of foraging within a food web more influenced by C4 photosynthesis than Nelson's Sparrows,

Table 1

Competing candidate generalized linear models compared using Akaike Information Criteria for small sample sizes (AIC_c). Models are designed to test multiple alternative hypotheses on the influence of species, breast feather $\delta^{15}\text{N}$ and breast feather $\delta^{13}\text{C}$ on breast feather mercury (Hg) concentrations in Saltmarsh, Seaside, and Nelson's Sparrows. 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 most parsimonious model); w_i (Akaike model weight); k (number of model parameters). Models with appreciable model support are shown in bold.

Model	AIC_c	ΔAIC_c	w_i	k
Hg = Species + $\delta^{15}\text{N}$ + Species \times $\delta^{15}\text{N}$	97.3	0.0	0.54	8
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	99.6	2.3	0.17	7
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Species \times $\delta^{15}\text{N}$	100.0	2.7	0.14	9
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Species \times $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	100.0	2.7	0.14	10
Species + $\delta^{15}\text{N}$	106.8	9.5	<0.01	5
Species + $\delta^{13}\text{C}$	107.9	10.6	<0.01	5
Species + $\delta^{13}\text{C}$ + Species \times $\delta^{13}\text{C}$	108.0	10.7	<0.01	8
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Species \times $\delta^{13}\text{C}$	109.2	11.9	<0.01	9
Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$	109.3	12.0	<0.01	6
$\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	145.5	48.2	0.000	4
$\delta^{13}\text{C}$	155.3	58.0	0.000	2
$\delta^{15}\text{N}$ + $\delta^{13}\text{C}$	156.5	59.2	0.000	3
$\delta^{15}\text{N}$	180.4	83.1	0.000	2
Species	586.1	488.8	0.000	4

Table 2

Well-supported models and accompanying generalized linear model statistics for breast feather Hg concentrations. Models were selected using AIC_c from candidate model sets (Table 1). All parameters refer to breast feather samples from Saltmarsh, Seaside, and Nelson's Sparrows captured during their non-breeding period (September through April) in NC salt marshes from 2006 to 2008.

Model	Source	df	F-value	P value	r^2
Hg = Species + $\delta^{15}\text{N}$ + Species \times $\delta^{15}\text{N}$	Overall	5	23.09	<0.01	0.36
	Species	2	4.42	0.01	
	$\delta^{15}\text{N}$	1	0.08	0.78	
	Species \times $\delta^{15}\text{N}$	2	5.67	<0.01	
Hg = Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	Overall	5	22.80	<0.01	0.35
	Species	2	30.28	<0.01	
	$\delta^{15}\text{N}$	1	9.39	<0.01	
	$\delta^{13}\text{C}$	1	10.35	<0.01	
Hg = Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	Overall	6	19.15	<0.01	0.36
	Species	2	4.27	0.02	
	$\delta^{15}\text{N}$	1	0.08	0.77	
	$\delta^{13}\text{C}$	1	0.00	0.96	
Hg = Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Species \times $\delta^{15}\text{N}$	Overall	7	16.62	<0.01	0.36
	Species	2	1.60	0.21	
	$\delta^{15}\text{N}$	1	1.34	0.25	
	$\delta^{13}\text{C}$	1	1.31	0.25	
Hg = Species + $\delta^{15}\text{N}$ + $\delta^{13}\text{C}$ + Species \times $\delta^{13}\text{C}$ + $\delta^{15}\text{N} \times \delta^{13}\text{C}$	Overall	7	16.62	<0.01	0.36
	Species	2	1.60	0.21	
	$\delta^{15}\text{N}$	1	1.34	0.25	
	$\delta^{13}\text{C}$	1	1.31	0.25	
Species \times $\delta^{13}\text{C}$	Species \times $\delta^{13}\text{C}$	2	1.12	0.33	
	$\delta^{15}\text{N} \times \delta^{13}\text{C}$	1	1.31	0.25	

Table 3

Relative importance of variables based on sums of Akaike weights (w_i) for each parameter in the candidate models (excluding models with non-informative parameters) testing multiple alternative hypotheses on the influence of species, breast feather $\delta^{15}\text{N}$ and breast feather $\delta^{13}\text{C}$ on breast feather mercury concentrations in Saltmarsh, Seaside, and Nelson's Sparrows.

Parameter	$\sum w_i$
Species	1.00
$\delta^{15}\text{N}$	1.00
Species \times $\delta^{15}\text{N}$	0.68
$\delta^{13}\text{C}$	0.46
$\delta^{15}\text{N} \times \delta^{13}\text{C}$	0.31
Species \times $\delta^{13}\text{C}$	0.14

which forage within a food web more dominated by C3 photosynthesis (Table 4).

4. Discussion

Breast feathers sampled in our study represent Hg exposure on unknown breeding sites for any given individual. Although little is known about the connectivity of breeding and non-breeding populations for any of these species, previous research allows us to hypothesize on factors affecting Hg exposure during the breeding period in these sparrows. The variables that we have identified as being important to breast feather Hg in this study may be driven by one or more inter-related ecological factors: (1) species may feed at a different trophic positions (Anderson et al., 2009; Jardine et al., 2006); (2) species may feed in environments with different levels of Hg bioavailability or contamination (Edmonds et al., 2010; Lane et al., 2011); (3) species may feed within food webs influenced to a greater or lesser extent by C4 and C3 photosynthesis (Cristol et al., 2011); and/or (4) species may feed within food webs with varying baseline $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures (Hobson, 1999; McClelland et al., 1997; Wigand et al., 2007). We address each of these factors with regard to our results below.

A study on breeding sites in Maine documented higher blood Hg concentrations in Saltmarsh compared to Nelson's Sparrows, and the authors hypothesized that this difference was due to differential prey selection (Shriver et al., 2006). However, to our knowledge, no comparative study of trophic position during the breeding season exists for these species. During the non-breeding period, Saltmarsh, Seaside, and Nelson's Sparrows exhibited similar blood $\delta^{15}\text{N}$ signatures (indicative of recent dietary exposure) at NC sites (Michaelis, 2009), and Cristol et al. (2011) documented similar results for blood $\delta^{15}\text{N}$ signatures in Saltmarsh and Nelson's Sparrows in Virginia. In both of these instances, Saltmarsh Sparrows had higher blood Hg concentrations compared to other sparrows on shared sites (Cristol et al., 2011; Michaelis, 2009; Winder and Emslie, 2011b), indicating differential Hg exposure unrelated to trophic position.

In the absence of diet data specific to the breeding season, information on winter diet serves as the only available starting point for our interpretation of $\delta^{15}\text{N}$ and Hg signals representative of breeding season diet. Consequently, it seems likely trophic position is not the

Table 4

Sample sizes and means \pm SE (ranges) for $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ (‰) and mercury (Hg ppm fw) in breast feathers sampled from Saltmarsh, Seaside, and Nelson's Sparrows in NC salt marshes during the non-breeding season from 2006 to 2008.

Species	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		Hg	
	n	Ratio	n	Ratio	n	Concentration
Saltmarsh Sparrow (<i>Ammodramus caudacutus</i>)	65	11.5 \pm 0.2 ^a (6.9 to 16.4)	65	-13.5 \pm 0.3* (-24.8 to -10.4)	59	5.4 \pm 0.4* (1.0 to 14.9)
Seaside Sparrow (<i>A. maritimus</i>)	83	11.4 \pm 0.2* (8.7 to 17.0)	83	-13.9 \pm 0.3* (-23.7 to -11.2)	72	2.8 \pm 0.3 ^{***} (0.6 to 12.6)
Nelson's Sparrow (<i>A. nelsoni</i>)	95	10.6 \pm 0.3 ^{**} (5.9 to 18.4)	95	-21.9 \pm 0.5 ^{**} (-29.5 to -12.2)	88	1.8 \pm 0.2 ^{***} (0.1 to 12.0)

^a Asterisks within columns for each variable indicate significant differences among species (P<0.05; using generalized linear models with Tukey multiple comparison tests).

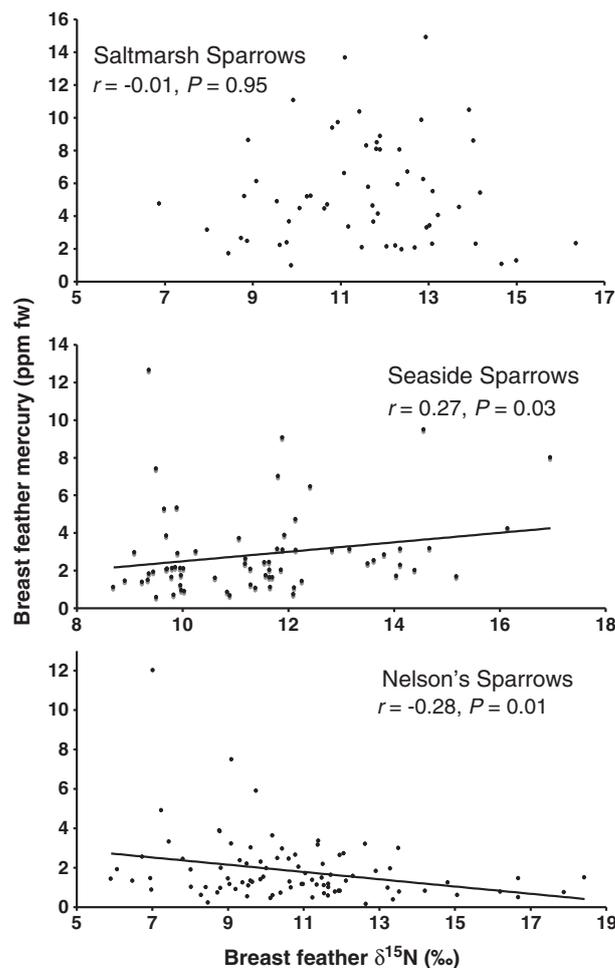


Fig. 1. Associations between breast feather Hg (ppm fw) and $\delta^{15}\text{N}$ (‰) in Saltmarsh, Seaside, and Nelson's Sparrows (*Ammodramus caudacutus*, *A. maritimus*, and *A. nelsoni*, respectively). Birds were captured during the non-breeding season in NC salt marshes from 2006 to 2008. Correlation coefficients, P-values and solid lines through data points represent strength of associations between these two variables using simple Pearson correlations. Linear regression analyses were not applied to these data; lines are for graphical display only.

main factor in determining Hg exposure in these species at either shared or separate sites. Therefore, the associations between $\delta^{15}\text{N}$ and Hg that we observed in breast feathers (Fig. 1) may be based more on geographic variation in $\delta^{15}\text{N}$ baselines from either natural variability or anthropogenic input (Hobson, 1999; McClelland et al., 1997; Wigand et al., 2007) than they are reflective of the influence of trophic position on Hg exposure. However, additional research on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ signatures and blood Hg in breeding individuals is necessary to test this hypothesis.

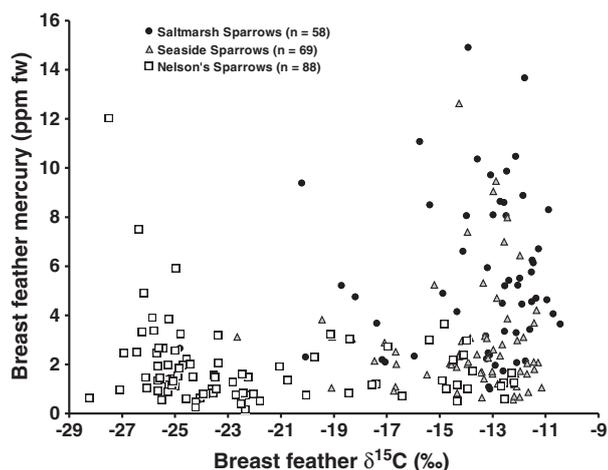


Fig. 2. Breast feather Hg (ppm fw) and $\delta^{13}\text{C}$ (‰) in Saltmarsh, Seaside, and Nelson's Sparrows (*Ammodramus caudacutus* (black circles), *A. maritimus* (gray triangles), and *A. nelsoni* (white squares), respectively). Birds were captured during the non-breeding season in NC salt marshes from 2006 to 2008. No species exhibited a significant correlation between breast feather Hg and $\delta^{13}\text{C}$ ($r < 0.12$, $P > 0.37$).

Differences in Hg exposure among breeding sites have been documented for both Saltmarsh and Nelson's Sparrows (Lane et al., 2011; Winder and Emslie, 2011a) as well as for other songbird species (Edmonds et al., 2010). Thus, some amount of variation in breast feather Hg (both within and among species) is likely due to geographic variation in Hg contamination/bioavailability on breeding sites. However, geographic variation in Hg exposure may coincide with shifting $\delta^{15}\text{N}$ baselines among sites (Nisbet et al., 2002). For this reason, we cannot determine whether the positive and negative associations we observe between $\delta^{15}\text{N}$ and Hg in Seaside and Nelson's Sparrows, respectively (Fig. 1), are indicative of true intra-specific variation in trophic position and related Hg exposure, or whether they represent geographic variation in Hg contamination/bioavailability and $\delta^{15}\text{N}$ baselines.

The decoupling between $\delta^{15}\text{N}$ and Hg we observed in Saltmarsh Sparrows may also be geographically driven. The breeding range of Saltmarsh Sparrows encompasses an area of North America that is especially vulnerable to Hg contamination (Evers et al., 2005; Rimmer et al., 2005), and blood Hg concentrations in this species have been found to vary significantly among sites (Lane et al., 2011). Site-to-site variability in Hg exposure for Saltmarsh Sparrows would increase the variation in tissue Hg concentrations within a given trophic position and thereby reduce our ability to detect any signal of trophic effect among the noise. Alternatively, decoupling of $\delta^{15}\text{N}$ and Hg in Saltmarsh Sparrows may be due to temporal factors. Thompson et al. (1998) reported decoupled $\delta^{15}\text{N}$ and Hg in body feathers of Northern Fulmars (*Fulmarus glacialis*) and Great Skuas (*Catharacta skua*) and attributed this result to $\delta^{15}\text{N}$ and Hg reflecting diet over different time scales. Blood Hg data from several studies indicate that Saltmarsh Sparrows are consistently exposed to comparatively higher levels of Hg than either Seaside or Nelson's Sparrows via an as yet unconfirmed mechanism (Cristol et al., 2011; Shriver et al., 2006; present study). This difference in Hg exposure could result in a cumulative body burden of Hg in Saltmarsh Sparrows that exceeds the amount of Hg that can be excreted into growing feathers, causing an increase in Hg body burden over time as net intake exceeds net excretion. If this were occurring within any portion of the population, breast feather Hg would not be representative of diet prior to molt in those individuals but of a growing body burden of Hg (including, but not limited to recent diet). This possibility highlights the need for further study of blood Hg dynamics in this species, especially since Hg levels in some populations of breeding Saltmarsh Sparrows are known to be high enough to warrant concern about their possible negative effects (Lane et al., 2011).

Our results indicate Nelson's Sparrows forage within C3-dominated food webs during the breeding season, while Saltmarsh and Seaside Sparrows forage within C4-dominated food webs. This result is consistent with the species biology as Nelson's Sparrows breed in habitats that are generally more dominated by C3 vegetation than breeding habitats for Saltmarsh Sparrows which are comprised largely of the C4 grass, *Spartina* (Greenlaw and Rising, 1994). $\delta^{13}\text{C}$ ranks with moderate importance in our candidate models, indicating that the influence of C3 versus C4 photosynthesis has some predictive power for explaining tissue Hg. Cristol et al. (2011) suggest that sparrows foraging on shared sites within different food web structures could be exposed to significantly different quantities of Hg based on microhabitat variation in mercury methylation and subsequent bioavailability. Our data support this explanation but require its extension to a much larger geographic scale. We contend that variation in Hg exposure across such a wide geographic area is likely also influenced by differential and site-specific Hg contamination/bioaccumulation (Edmonds et al., 2010; Lane et al., 2011; Winder and Emslie, 2011a).

5. Conclusions

Our study documents that species, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ influence breast feather Hg concentrations in Saltmarsh, Seaside, and Nelson's Sparrows. Though our top four models represent 99% of the total support in our candidate model set, those models explain only a modest proportion of variability in breast feather Hg with r^2 values ranging from 0.35 to 0.36. Obviously unidentified factors are contributing to breast feather Hg. 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. Additionally, a study of diet and Hg exposure during the breeding season for each of these species is necessary to determine if geographic variability in Hg exposure (from regional to microhabitat scales) may be the driving force behind species differences in Hg concentrations (Cristol et al., 2011).

We have presented novel information on associations between Hg and $\delta^{15}\text{N}$ for a tissue in which both of these dietary signals should represent breeding season diet. We have found no evidence that Hg exposure in Saltmarsh, Seaside, and Nelson's sparrows is related to trophic position despite $\delta^{15}\text{N}$ being a supported variable in our models. Instead, geographic variation may well be the most important driver of Hg exposure in these sparrows and is in need of additional investigation.

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