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# Mercury levels of Nelson's and saltmarsh sparrows at wintering grounds in Virginia, USA

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**Abstract** Nelson's and saltmarsh sparrows (*Ammodramus nelsoni* and *A. caudacutus*) have recently been recognized as separate species, and because of their limited distributions and the susceptibility of their wetland habitats to climate change, these two new species are of conservation concern. Both species are known to bioaccumulate mercury at breeding sites in New England, USA where their ranges overlap, with the saltmarsh sparrow reported to have twice the concentration of blood total mercury. In this study we sampled both species on their shared wintering grounds, and documented that mercury exposure is lower than that reported for the breeding range, with saltmarsh sparrow blood mercury 2.6 times higher than in Nelson's sparrow. Feather mercury, which is incorporated on the breeding grounds, confirmed that saltmarsh sparrows had incorporated 2.3 times more mercury than Nelson's sparrows during the previous breeding season. A comparison of stable isotopes of nitrogen and carbon suggests that the higher exposure of saltmarsh sparrows may be not due to feeding at a higher trophic level, as previously hypothesized, but rather could be related to a difference in the carbon source at the base of each species' food chain. This study, along with recently published data from both species on additional breeding and wintering grounds, provides a more complete picture of relative mercury exposure. Saltmarsh sparrows are exposed to mercury levels that warrant concern, with the highest exposure being during the breeding season. Areas set aside for the long-term conservation of this species should be carefully assessed for mercury bioaccumulation.

**Keywords** Chesapeake Bay · Mercury · Nelson's sparrow · Saltmarsh sparrow · Stable isotopes · Wintering ground

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## Introduction

Nelson's and saltmarsh sparrows (*Ammodramus nelsoni* and *A. caudacutus*) have recently been recognized as distinct species (American Ornithologists' Union 1995), and because of their limited distributions and the vulnerability of their wetland habitat to climate change, both are species of conservation concern (Dettmers and Rosenberg 2000; IUCN 2010; Rich et al. 2004; U.S. Fish and Wildlife Service 2002). Nelson's sparrows breed in three distinct ranges, all in northern North America: Great Plains, Hudson Bay, Maritimes. Saltmarsh sparrows nest only in coastal tidal marshes from northern New England south to Virginia, with Chesapeake Bay being the southern limit of their current breeding range (Watts 2005). Both species winter together in tidal marshes along the east coast of North America (Greenlaw and Woolfendon 2007), including the site of this study, Chesapeake Bay and adjacent seaside marshes of the Delmarva Peninsula, Virginia.

Mercury is a mobile pollutant that can alter immune and endocrine profiles in songbirds, and affect reproductive success and survival (Brasso and Cristol 2008; Custer et al. 2007; Hallinger et al. 2011; Scheuhammer et al. 2007). Species that eat wetland invertebrates are vulnerable to mercury exposure because mercury becomes more

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**Table 1** Rangewide saltmarsh and Nelson's sparrow mercury (Hg) concentrations (ppm wet weight)

| Location | Blood Hg (*n*) (range[a]) | Feather Hg (*n*) (range[a]) | Sampled | Reference |
|---|---|---|---|---|
| Ontario | 0.22 (13) (0.14–0.36) | 1.21 (14) (0.47–5.72) | Breeding Nelson's | Winder and Emslie, 2011 |
| North Dakota | 1.07 (24) (0.68–1.87) | 0.98 (24) (0.34–3.19) | | |
| Maine | 0.69 (53) (0.56-0.87) | NA | Breeding saltmarsh | Shriver et al. 2006 |
| | 0.41 (28) (0.26–0.56) | NA | | |
| | 0.64 (229) (0.31–0.85) | NA | | Lane et al., in press |
| New Hampshire | 0.74 (95) (0.32–1.10) | NA | | |
| Massachusetts | 1.37 (160) (0.88–1.80) | NA | | |
| Rhode Island | 0.80 (81) (0.59–1.10) | NA | | |
| Connecticut | 0.50 (31) (0.24–0.61) | NA | | |
| New York | 1.01 (44) (0.68–1.50) | NA | | |
| Delaware | 0.48 (35) (0.40–0.54) | NA | | Warner, 2009 |
| Virginia | 0.14 (130) (0.09–0.20) | 2.76 (114) (1.81–4.74) | Wintering Nelson's | This study |
| | 0.37 (127) (0.15–0.68) | 6.25 (105) (2.96–10.76) | Wintering saltmarsh | |
| North Carolina | 0.14 (47) (0.11–0.16) | 2.94 (55) (2.57–3.80) | Wintering Nelson's | Winder and Emslie, 2011 |

[a] Sample size represents individuals sampled. Range, for North Dakota and Ontario, where a single site was sampled, represents minimum and maximum individual values. At other locations multiple sites were sampled and range is minimum and maximum of site means

**Table 2** Locations of sample collections in Virginia, USA

| Site # | Location name | Latitude | Longitude |
|---|---|---|---|
| 1 | Assateague Bay | 37.954654 | −75.317580 |
| 2 | Chincoteague | 37.922407 | −75.350723 |
| 3 | Smalley Drain | 37.912024 | −75.367448 |
| 4 | Tom's Cove | 37.891455 | −75.353260 |
| 5 | Belinda | 37.905207 | −75.685916 |
| 6 | Parramore Island | 37.572239 | −75.614844 |
| 7 | Magotha | 37.174903 | −75.943232 |
| 8 | Bull's Drive | 37.142255 | −75.941172 |
| 9 | Fishermans Island | 37.094062 | −75.967886 |
| 10 | Poquoson | 37.111965 | −76.325995 |
| 11 | Maryus | 37.277605 | −76.410803 |
| 12 | Monday Creek | 37.282993 | −76.385990 |

bioavailable when methylated by wetland microbes, and can biomagnify up aquatic or terrestrial food chains (Cristol et al. 2008). Both Nelson's and saltmarsh sparrows accumulate mercury at breeding sites where their ranges converge in northern New England (Shriver et al. 2006), with blood total mercury concentrations in saltmarsh sparrows being nearly twice as high as those in Nelson's (0.7 ppm versus 0.4 ppm, Tables 1, 2). Another study found elevated mercury in Nelson's sparrows breeding in North Dakota (1.1 ppm, Table 1). Thus, some breeding populations of both species appear to have elevated mercury exposure, which could serve as an additional stressor in species already of conservation concern.

The impact of mercury on avian health is likely due to the seasonal duration of exposure as well as level and form of mercury. Migratory birds that move to areas with lower mercury should be able to reduce mercury load through internal detoxification (e.g. hepatic demethylation and sequestration, Henny et al. 2002), or molt of feathers, as feathers concentrate a large proportion of body burden and replacement feathers remove a substantial amount of mercury from the rest of the body (Condon and Cristol 2009). Thus, the impact of mercury on Nelson's and saltmarsh sparrows will be influenced by their degree of mercury exposure during the 6–8 months spent on the wintering grounds. The objective of this study was to (1) measure mercury levels in blood and feathers of both species on a portion of the wintering range where they co-occur, and (2) test the hypothesis that differences in mercury exposure during winter are due to differences in diet as reflected in stable isotope signatures.

## Materials and methods

### Study species

Nelson's and saltmarsh sparrow were formerly lumped as sharp-tailed sparrow until 1995 so researchers have only recently focused on their differences (American Ornithologists' Union 1995). Both species are difficult to observe and little has been published on their habitats, diets or contaminant profiles (Shriver et al. 2010). Nelson's sparrow has three distinct breeding populations in (1) northern Great Plains (*A. n. nelsoni*), (2) Hudson Bay (*A. n. alterus*), and (3) Canada/USA maritime coast (*A. n. subvirgatus*). Saltmarsh sparrows breed only on the east coast of the

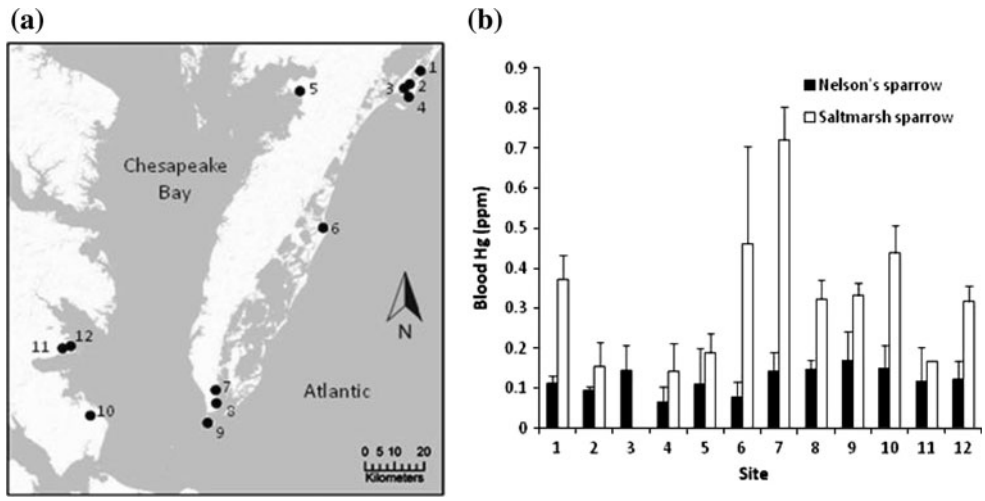


**Fig. 1** **a** Sampling locations and **b** geometric mean of blood total mercury levels for Nelson's sparrows (*black bars*) and saltmarsh sparrows (*white bars*). Error bars are 95% confidence intervals. *Numbers* refer to sites and are described in Table 2

USA from Maine south to Virginia (Watts 2005). There are two distinct subspecies, *A. c. caudacutus* and *A. c. diversus*, with the dividing line of breeding ranges being in New Jersey (Greenlaw and Rising 1994). In this study we sampled multiple subspecies of both species near the northern edge of their shared winter range in the tidal marshes of the Chesapeake Bay, and adjacent seaside marshes of the Delmarva Peninsula, Virginia, USA. Our sampling was undertaken outside of the breeding range for all but one subspecies, *A. c. diversus*. However, this species is not a common breeder at the sampled sites and we believe that nearly all sparrows sampled for this wintering ground study were migrants from other breeding grounds.

## Sample collection

Sparrows were captured during the wintering period from 17 December, 2008 through 25 February, 2009 and 25 October, 2009 through 7 April, 2010 at 12 sites chosen based on likely presence of both species (Fig. 1a). Teams of 3–10 people walked abreast, dragging a 60 m weighted rope through appropriate habitat to flush birds into mist nets. Birds were removed from nets and up to 100 µl of blood was collected in two heparanized 75 µl capillary tubes from the brachial vein punctured with a 30 gauge needle. One tube was used for mercury analysis, and the other for stable isotope analysis. Blood was stored on ice for up to 8 h, and then frozen at −20°C until analysis. After morphometrics were recorded, birds were photographed and 5 breast feathers were plucked and frozen in zipped plastic bags.

## Sample preparation

Blood samples were thawed and expressed directly onto a balance before being transferred to the mercury analyzer; reported values are for wet weight. Feathers were thawed, washed with distilled water to remove particulates, dried for 48 h in a low-humidity chamber and analyzed for mercury; these are also reported as wet weights. Blood samples used for isotopic analysis were dehydrated on a Labconco freeze-drier for 24 h before homogenization and then packaged in crimped tin containers for shipping.

## Total mercury analysis

Samples were analyzed for total mercury at the College of William and Mary between 19 March, 2009 and 14 September, 2010. We used atomic absorption spectroscopy with a Milestone DMA-80 direct mercury analyzer (Shelton, CT, USA). The DMA-80 was calibrated using known standards according to machine specifications prior to the analysis and approximately every 2 months throughout the study period, or more often when necessary to keep standard reference material values within 7.5% of certified values. A blank, an empty sample container, a duplicate and two aliquots of each standard reference material (DORM-3 and DOLT-4) were run with every 20 samples. Two separate capillary tubes of blood from the same collection date of the same bird run on the same day were considered duplicate blood samples ($n = 11$ pairs of samples). Due to a paucity of sample duplicates, pairs of avian blood samples from a similar study on tree swallows (*Tachycineta bicolor*) were run as additional duplicates for this study ($n = 13$ pairs of samples). Likewise, swallow wing feathers were cut into 1 $mm^2$ pieces, homogenized for 1 min, and used in paired aliquots for duplicate feather samples ($n = 16$ pairs of samples). Thus, duplicates were run for 6.3% of feather samples and 9.3% of blood samples. Relative percent difference between duplicate blood samples was 11.4 ± 10.8% (mean ± SD reported throughout text), and 7.2 ± 6.8% for feathers. Minimum

detection limit was 0.002 to 0.009 ppm over the entire period of the study and all samples were above detection limit. Recovery of total mercury was 95.9% ± 3.6% for DORM-3 ($n = 38$) and 94.9 ± 2.1% for DOLT-4 ($n = 38$). All other quality control measures were within acceptable limits.

### Stable isotope analysis

Blood for stable isotope analysis was collected simultaneous with mercury samples and analyzed at the University of California-Davis Stable Isotope Facility (Davis, CA, USA). Ratios of stable isotopes of nitrogen and carbon were measured by continuous-flow isotope ratio mass spectrometry (20–20 mass spectrometer, Sercon, Crewe, UK). The samples were combusted at 1,000°C in an on-line elemental analyzer (PDZEuropa, Sandbach, UK; ANCA-GSL, Cheshire, UK). Sample ratios were compared to those of pure cylinder gases injected into the spectrometer before and after the sample peaks. Stable isotope ratios are reported in parts per thousand (‰), in the standard delta ($\delta$) notation, of the standards for nitrogen (atmospheric nitrogen) and carbon (Vienna PeeDee Belemnite). The equation, $\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 100$, was used to calculate values, where $X$ is the heavier isotope, $^{15}$N or $^{13}$C; $R_{\text{sample}}$ is the isotopic ratio in the sample; and $R_{\text{standard}}$ is the ratio in the standard. Measurement precision of within-run standards for $\delta^{15}$N and $\delta$ $^{13}$C was ±0.1‰.

### Data analysis

Mercury measurements were log transformed prior to statistical analyses. Geometric means with 95% confidence intervals are reported for all mercury measures. Comparisons of mercury and isotopes for the two species were made using a two-tailed $t$-test. Relationships between feather and blood mercury, and mercury and $\delta^{15}$N, were tested using linear regressions. A general linear model with species, site, and their interaction as factors was used to investigate differences in mercury between sampling sites. All statistics were performed in SPSS 19 (IBM).

## Results

Mercury levels in blood for all birds combined were very similar across the two winters of the study (2008–2009: 0.18 ± 0.05 ppm, $n = 77$, 2009–2010: 0.20 ± 0.03 ppm, $n = 178$). As there was no significant difference between years they were combined for all analyses (year: $F_{1,\ 254} = 1.45$, $P = 0.23$; year × species interaction $F_{1,\ 251} = 5.46$, $P = 0.02$). Both species and site significantly affected blood mercury concentration (Whole model $r^2 = 0.71$; Species: $F_{1,\ 235} = 94.10$, $P < 0.01$; Site: $F_{8,\ 228} = 15.45$, $P < 0.01$; site*species interaction: $F_{8,\ 219} = 8.90$, $P < 0.01$) such that saltmarsh sparrows had higher mercury than Nelson's sparrows at every site and absolute mercury levels varied across sites (Fig. 1b).

Total mercury in blood of saltmarsh sparrows on wintering sites was 0.33 ± 0.21 ppm ($n = 124$), and was approximately 3.0× higher than that of Nelson' sparrows captured at the same sites and season (0.11 ± 0.08 ppm, $n = 131$). Feather mercury was also approximately 2.6× higher in saltmarsh sparrows (5.42 ± 0.65 ppm, $n = 125$) than in Nelson's sparrows (2.08 ± 0.40 ppm, $n = 143$; $F_{1,\ 267} = 115.04$, $P < 0.001$). The relationship between each individual's feathers and blood was weak in both species, but significant and positive in saltmarsh sparrows (saltmarsh: $n = 101$, $r^2 = 0.06$, $F = 6.13$, $P = 0.02$; Nelson's: $n = 108$, $r^2 = 0.02$, $F = 2.06$, $P = 0.15$; Fig. 2).

Stable isotope analyses revealed that saltmarsh sparrows had similar $\delta^{15}$N values (10.458 ± 1.348, $n = 92$) to Nelson's sparrows (10.672 ± 1.896 ‰, $n = 90$, $t = 0.88$, $P = 0.38$; Fig. 3), whereas the two species differed in $\delta^{13}$C (saltmarsh: −16.238 ± 2.100‰, $n = 92$; Nelson's: −13.859 ± 1.885, $n = 90$; $t = 8.04$, $P < 0.01$, significant with Bonferroni adjustment for multiple tests; Fig. 3). There was a weak but significant positive relationship between individual $\delta^{15}$N and blood mercury levels in both species (saltmarsh: $n = 78$, $r^2 = 0.11$, $F = 9.184$, $P < 0.01$; Nelson's: $n = 78$, $r^2 = 0.12$, $F = 10.43$, $P < 0.01$; Fig. 4).

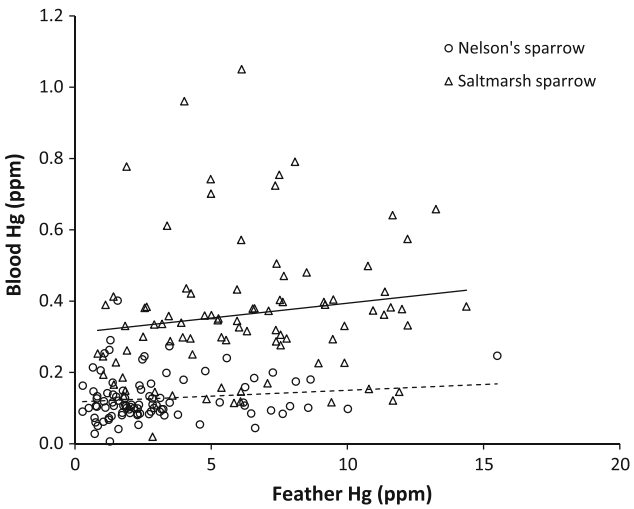


**Fig. 2** Correlations between paired blood and feather mercury levels from the same individual for Nelson's sparrow (*open circle*) and saltmarsh sparrow (*open triangle*) sampled in Virginia wintering range. *Lines* are linear regressions for Nelson's sparrow (*dashed*) and saltmarsh sparrow (*solid*)

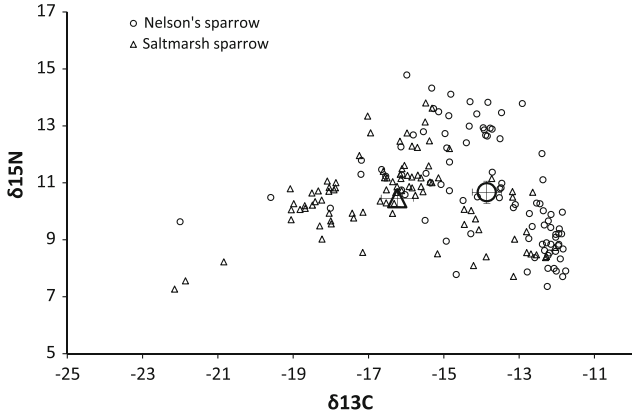


**Fig. 3** Stable isotope values for Nelson's sparrow (*open circle*) and saltmarsh sparrow (*open triangle*). *Small symbols* are individual measures, *large symbols* are species average values with error bars representing the 95% confidence interval

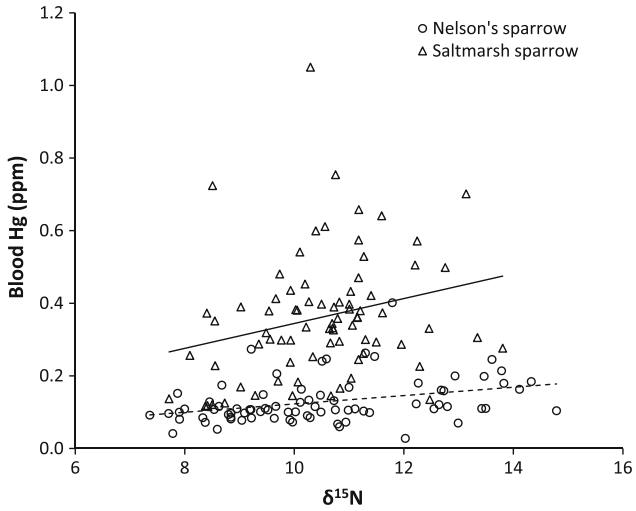


**Fig. 4** Correlations between nitrogen stable isotope enrichment and mercury level for Nelson's sparrow (*open circle*) and saltmarsh sparrow (*open triangle*). *Lines* are linear regressions for Nelson's sparrow (*dashed*) and saltmarsh sparrow (*solid*)

## Discussion

Saltmarsh and Nelson's sparrows have been recognized as separate species only since 1995 and thus biologists are now describing the unique natural histories of each species (Greenlaw and Rising 1994). Interest in their mercury levels began in 2006 with the report that where the breeding range of the two overlapped, in Maine, USA, saltmarsh sparrows had higher blood mercury concentrations than Nelson's sparrows, thought to be due to their larger bill allowing them to forage at a higher trophic level (Shriver et al. 2006). Extensive sampling since that time has revealed that at many breeding sites saltmarsh sparrows bioaccumulate mercury in blood to levels above those typically believed to harm songbirds (Lane et al., in press). A recently published study on Nelson's sparrows documented that at one of two previously unstudied inland sites, blood mercury levels are comparable to saltmarsh sparrow levels (North Dakota), whereas at the other site (Ontario) levels are below those originally reported for Nelson's sparrows in Maine (Shriver et al. 2006). Thus, some populations of both species experience mercury levels of concern during the breeding season. Unfortunately, there is not yet enough information about the effects of mercury on free-living songbirds to determine the appropriate level of concern for breeding population of these two vulnerable species.

Both sparrow species are migratory, wintering together at numerous tidal marshes along the eastern coast of North America as well as the Gulf of Mexico. We sampled the northern portion of the shared wintering range, in the Chesapeake Bay and seaside marshes of the Delmarva Peninsula, Virginia, and found that while both species had lower blood mercury than on their breeding ranges, the relative difference was the same as originally reported for their shared breeding range, with saltmarsh sparrows having approximately 2.5 times the concentration of blood mercury. Feathers incorporate mercury from blood at the time that they are grown, thus the feathers we sampled on the winter range contained mercury from the breeding range (Condon and Cristol 2009). Not surprisingly, then, there was only a weak relationship between feather and blood mercury within individuals of either species, but the relative difference between the species was similar to that reported for blood on the shared breeding range (Shriver et al. 2006). Feather and blood mercury values of Nelson's sparrows from North Carolina wintering sites were recently published (Winder and Emslie 2011), and are remarkably similar to the values presented here (Table 1).

These results suggest that saltmarsh sparrows, which nest along the northeastern coast of North America, lower their mercury levels when they migrate to wintering grounds along the southeastern coast. Several breeding populations have mercury levels that warrant concern (Lane et al., in press), particularly when considering the other pressures on this species (e.g. coastal "squeeze" due to real estate development and climate-induced sea level rise) and the vulnerability of embryos to mercury deposited by females exposed during the breeding season. However, for the majority of the year, this species experiences mercury levels that are lower than on the breeding ground. This may be due to a shift in diet downwards on the food chain, for example fewer insects and more seeds. It may also reflect lower environmental mercury levels. Bald eagles (*Haliaeetus leucocephalus*) have lower feather mercury levels in the Chesapeake Bay than do those in any other

North American population thus far reported (D. Cristol, unpublished data). Regardless of the mechanism, saltmarsh sparrow blood mercury is 2–3× higher on the breeding grounds (Table 1) and this is where any effort at ameliorating the mercury exposure threat should be focused.

In contrast to saltmarsh sparrows, Nelson's sparrows nesting in eastern North America (*A. n. alterus and A. n. subvirgatus*) appear to face little threat from mercury exposure, based on currently available data. However, the central population (*A. n. nelsoni*), which was recently sampled in North Dakota, deserves a closer look (Table 1). The elevated levels (1.1 ppm, Table 1) reported from 24 individuals at a single site are cause for alarm, but conclusions about this population require more expansive sampling throughout the large breeding range in the Great Plains of Canada and USA.

While mercury levels of Nelson's sparrows on the wintering grounds may have no immediate implications for conservation of this species, due to their generally low concentrations year-round, they are interesting when compared to the higher levels in the closely-related saltmarsh sparrow. One hypothesis for the consistent difference between the two species is that saltmarsh sparrows, with their larger bill, eat larger prey that occupy a higher trophic position, and thus experience more biomagnification of mercury (Shriver et al. 2006). To test this hypothesis, we compared signatures of stable isotopes of nitrogen, a commonly used proxy for trophic position (Kelly 2000). The two species had very similar nitrogen signatures in blood sampled on the wintering ground, suggesting that during the non-breeding season they eat diets that are equivalent in terms of trophic position. It should be noted, however, that estimating relative trophic position from nitrogen signatures assumes that both species are feeding on the same food web, which is an open question (see below).

In contrast, saltmarsh sparrows were relatively enriched in the heavier carbon isotope, suggesting reliance on a food web with more plants that use C4 photosynthesis, such as *Spartina* grasses, as opposed to plants using C3 photosynthesis, such as *Juncus* rushes or woody shrubs. While little is known about habitat segregation in these two cryptic sparrow species, the difference in carbon stable isotopic signatures suggests that they sit atop different food chains in winter, at the base of which are different plants. This could occur in the same microhabitat through direct consumption of different seed species. The difference in carbon stable isotope signatures could also arise through habitat segregation leading to consumption of different seeds or invertebrates that have previously eaten different types of plants. Spatial segregation could be the direct explanation for the higher mercury level of saltmarsh sparrows, if, for example, more methylation occurs in sections of saltmarsh covered with *Spartina* than *Juncus* and shrubs. Alternately, the species' food chains may have differential terrestrial versus marine inputs. Further study will be necessary to link mercury to sparrow diet components and to track those to their source in the habitat.

In summary, saltmarsh sparrows have higher mercury levels than Nelson's sparrows on the wintering ground, as previously reported for the breeding grounds. The difference in mercury levels is not easily explained by feeding at different trophic levels, as previously suggested; rather, they may feed from food webs with different carbon sources, as would occur if they segregated between high (shrubby) and low (grassy) saltmarsh in winter. Saltmarsh sparrows, which face a potential threat from elevated mercury levels on the breeding grounds, have lower mercury after they migrate to wintering grounds. However, mercury is still present in both species on the wintering grounds and all populations of these two newly-recognized species of conservation concern should be monitored periodically as global mercury levels change.

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