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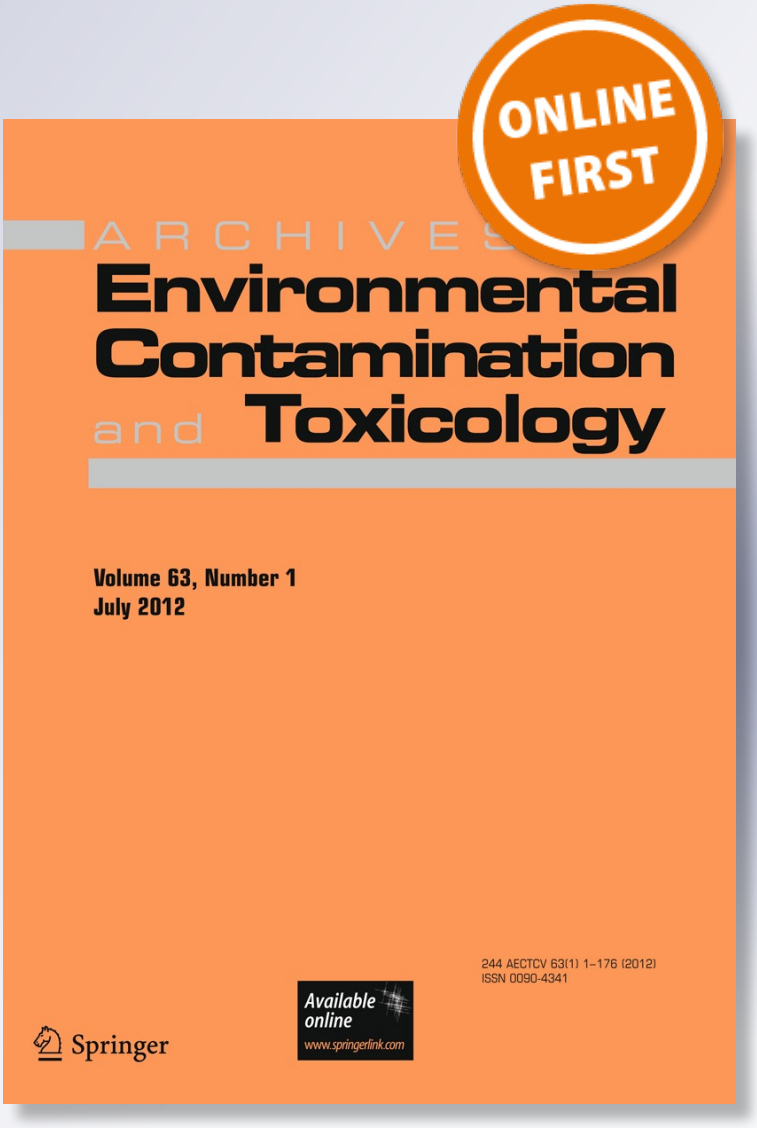

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Abstract We estimated mercury exposure and bioaccumulation in sparrow feathers to determine variation among age groups, between sparrow species, and between feather types. Results of feather mercury studies in piscivorous birds indicate that mercury concentrations tend to increase with age and differ between feather types; however, data for insectivorous birds are lacking. We estimated mercury exposure of two insectivorous and sympatric tidal marsh sparrows: coastal plain swamp sparrow (*Melospiza georgiana nigrescens)*, and seaside sparrow (*Ammodramous maritimus*). Tidal marshes have favorable conditions for mercury methlyation, thus it is likely that tidal marsh sparrows are exposed to methylmercury. We found no difference in mercury concentrations between males and female birds of both species. Adult swamp sparrow feather mercury concentrations did not differ among adult age groups; therefore, mercury was not found to increase with age in sparrows at the site. Hatch-year birds had significantly greater feather mercury concentrations compared with adult birds for both species. Mercury concentrations in adult seaside sparrows were twice as high as those in adult swamp sparrows suggesting species-specific variation, although concentrations in hatch-year sparrow species did not differ. Mercury concentrations differed between feather types in adults of both species. The first primary feather of both species had at least three times greater mercury concentrations than the outer tail feather possibly reflecting varying depuration rates with feather type.

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Tidal marsh sparrows are threatened by habitat loss and fragmentation from shoreline development and increasing sea levels, alterations of habitat by invasive species, management actions, and environmental toxins, such as mercury. The bioavailable form of mercury, methylmercury, is biologically harmful because it is readily absorbed by many organisms and bioaccumulates with continued exposure (Scheuhammer et al. 2007). High mercury concentrations in birds can alter biochemical processes, cause histopathological effects, affect immune system functions (Hoffman et al. 2009), and inhibit neurological function and body growth (Heinz and Hoffman 2003); all of these are effects that can lead to decreased overall fitness. Tidal marsh sparrows may be exposed to mercury due to the unique habitat characteristics of tidal marshes coupled with inputs of mercury from sediment loading and atmospheric deposition (Kongchum et al. 2006; Mitchell and Gilmour 2008; Orson et al. 1992).

Tidal marsh air and water temperature and sediment chemistry (e.g., anaerobic bacterial communities, high organic carbon and sulfate production) influence methylmercury

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production in the environment (Chen et al. 2008; Gilmour et al. 1992; Marvin-DiPasquale et al. 2003; O'Driscoll et al. 2011). Sparrows can be directly and indirectly exposed to mercury from dietary sources or by nesting in areas that have high levels of methylmercury production. Individuals that are unable to excrete methylmercury as fast as it is being assimilated will accumulate the heavy metal in tissues as they age (Rimmer et al. 2005). Birds do have the ability to depurate mercury, predominantly through feather molt, and concentrations of mercury in body tissues (e.g., muscle, liver, blood) correlate with those in feathers (Agusa et al. 2005; Burger 1993; Rattner et al. 2008; Thompson 2001; Thompson and Furness 1989; Zolfaghari et al. 2007). Mercury is incorporated into feathers as methylmercury, and once feather growth is complete the concentration of methylmercury in the feather is stable (Thompson and Furness 1989). Therefore, analysis of feathers is used as a noninvasive metric for estimating methylmercury exposure in birds at the time of feather molt (Burgess and Meyer 2008; Evers et al. 2008; Monteiro and Furness 1995; Spalding et al. 2000).

Few studies have investigated bioaccumulation and feather mercury dynamics in insectivorous species that occupy high methylmercury-production areas. Moreover, where such dynamics have been studied, the age of sampled individuals and feather types are often unknown or unreported (Bortolotti 2010). There are considerable data describing mercury concentrations relative to feather type and bird age in piscivorous birds. In some studies, molt sequence explains differences in mercury concentrations among feather types (Altmeyer et al. 1991), and bioaccumulation accounts for increases in mercury concentrations as individuals age (Evers et al. 2005). More information is needed to determine the relationships among these parameters to refine estimates of mercury dynamics in insectivorous birds. To that end, we estimated mercury exposure of two insectivorous tidal marsh sparrows: coastal plain swamp sparrow (*Melospiza georgiana nigrescens)* hereafter swamp sparrow, and seaside sparrow (*Ammodramous maritimus*) breeding in the same tidal marsh along Delaware Bay. Both sparrow species breed and forage exclusively in tidal marsh habitats (Mowbray 1997; Post and Greenlaw 2009). Previous research at our study site has determined mercury concentrations in sparrow blood to be greater than those in sparrows from other sites along the Delaware Bay (Warner et al. 2010); therefore, our study site proved optimal to investigate the baseline dynamics of mercury in feathers between two tidal marsh species that inhabit the same marsh. Our objectives were to (1) determine differences in feather mercury concentrations between males and female birds within each sparrow species, (2) examine bioaccumulation among adult swamp sparrows of different ages, (3) compare feather mercury levels of juvenile (hereafter "hatch-year") sparrows with those in adult birds, (4) compare mercury concentrations between the two sparrow species, and (5) compare mercury levels between primary and tail feathers on the same adult individual.

Materials and Methods

To determine concentrations of mercury in sparrows, we collected feathers from adult swamp and seaside sparrows during mid-breeding season (June to July 2007 and 2008). Feathers were sampled from adults before the initiation of the prebasic molt and from hatch-year birds in juvenile plumage before their first prebasic molt. Prebasic molt for adults occurs on the breeding grounds for both sparrow species (Dwight 1900; Post and Greenlaw 2009). We captured sparrows using mist-nets and collected the outer rectrix (hereafter "tail feather") from previously banded sparrows by cutting the feather close at the calamus. For swamp sparrows, we banded individuals as nestlings or aged individuals based on plumage scoring (Olsen 2007), which allowed us to determine specific ages. From the total samples, we collected tail feathers from individuals in four known age classes: (1) hatch-year ($n = 19$), (2) 1–2 years old ($n = 9$), (3) 3–4 years old ($n = 14$), and (4) 5–7 years old ($n = 28$). For seaside sparrows, we collected tail feathers from hatch-year (n=9) and adult birds [after hatch-year ($n = 15$)], but we were not able to separate these samples into more specific age groups. To compare feather mercury levels between feather types in a given individual, we collected the innermost primary feather [primary feather molted first (hereafter "primary")] and the outermost tail feather (tail feather molted last) from 15 randomly selected adult birds from both species. All feathers were immediately placed unaltered in a labeled, dry envelope and stored in a dry location until they were shipped to Texas A&M Trace Element Research Laboratory, College Station, TX, for analysis.

Feather samples were either analyzed whole or as subsamples after homogenization with a Spex 6800 cryomill. Samples were analyzed for mercury by combustion/trapping/cold-vapor atomic absorption using United States Environmental Protection Agency Method 7473 (1998). Feather samples were not freeze dried or altered before analysis. Dry feather samples were weighed to the nearest 0.1 mg in tared, combusted nickel boats. The boats were then loaded into the autosampler carousel of a Milestone DMA 80 mercury analyzer and sequentially introduced into the instrument's combustion chamber. Samples were heated in a tube furnace at 850 °C under a stream of oxygen, and combustion products were passed through a catalyst and then through a gold-coated sand column where mercury

atoms were trapped. After thermal desorption, the oxygen gas stream carried mercury vapor through two atomic absorption cells that quantified mercury over the range of 0.001–0.700 μg. We used certified reference materials as standards to calibrate the instruments. Calibration was monitored after every 10 samples and at the end of the analysis by analyzing a check standard and a blank. Laboratory quality-control samples included a method blank, certified reference material, a duplicate sample, and a spiked sample with each batch of ≤20 samples. All concentrations are expressed as arithmetic means with SEs and in parts per million (ppm) on a dry-weight (dw) basis.

Feather mercury concentrations were compared with one-way analysis of variance (ANOVA) based on the following independent variables: (1) sex (species analyzed separately), (2) age groups within species (hatch-year *vs.* after hatch-year for seaside sparrows; hatch-year, 1–2 years, 3–4 years, and 5–7 years for swamp sparrows). We used Tukey post hoc test for swamp sparrows to determine which age groups differed. We used paired Student *t* test to determine differences in mercury concentrations between adult primary and tail feathers within the same individual. We used SPSS version 19 for all analyses and set $\alpha = 0.05$.

We collected all sparrows from the Woodland Beach State Wildlife Management Area, Kent County, Delaware (39°21′N, 75°33′W), from a 15-ha study plot established in 2003 (Olsen 2007). Salinity at the study site ranged from 1 to 10 ppt. Vegetation included *Schoenoplectus americanus* (bulrush), the shrubs *Iva frutescens* (high-tide bush) and *Baccharis halimifolia* (groundsel bush), and the salt marsh grasses *Spartina patens* (saltmeadow cordgrass), *Spartina alterniflora* (smooth cordgrass), and *Distichlis spicata* (saltgrass). Limited patches of *Phragmites australis* (common reed) and *Spartina cynosuroides* (big cordgrass) occurred on the borders of the plot.

Table 1 Age groups of *M. georgiana* and *A. maritimus* and tail feather mercury ppm (dw) levels at Woodland Beach State Wildlife Management Area, Delaware, USA, from 2007 to 2008

Species	0 (hatch-year)	1 (1–2 year old)	2 (3–4 year old)	3 (5–7 year old)
M. georgiana				
Minimum	0.54	0.19	0.05	0.17
Maximum	5.68	0.89	0.88	1.56
Mean (SE)	1.72 (0.28)[a]	0.41 (0.09)[b]	0.33 (0.05)[b]	0.54 (0.06)[b]
n	19	9	14	28
A. maritimus	HY	AHY		
Minimum	0.99	0.46		
Maximum	2.35	3.13		
Mean (SE)	1.65 (0.16)[a]	1.01 (0.19)[b]		
n	9	15		

Superscript footnote symbols "a" and "b" indicate values that are significantly different

Table 2 *M. georgiana* and *A. maritimus* outer tail feather mercury ppm (dw) levels in sampled adult birds at Woodland Beach State Wildlife Management Area, Delaware, USA, from 2007 to 2008

Mercury ppm (dw)	*M. georgiana*	*A. maritimus*
Minimum	0.05	0.46
Maximum	1.56	3.13
Mean (SE)	0.46 (0.04)[a]	1.01 (0.19)[b]
n	51	15

Superscript footnote symbols "a" and "b" indicate values that are significantly different

Results

We did not detect a difference in the tail feather mercury concentration between male and female swamp sparrows ($F_{1,49} = 0.07$, $P = 0.80$) or between male and female seaside sparrows ($F_{1,13} = 0.74$, $P = 0.41$); therefore, we combined sexes for all other analyses. Swamp sparrow tail feather mercury differed among the four age groups ($F_{3,66} = 16.0$, $P < 0.001$). Swamp sparrow hatch-year birds had significantly greater mercury levels (on average four times greater) than adult age groups ($P < 0.001$); however, there was no difference among the adult age groups ($P > 0.05$; Table 1). Seaside sparrow hatch-year birds had 1.6 times greater mercury than adult seaside sparrows ($F_{1,22} = 5.6$, $P = 0.03$; Table 1).

We detected differences in mercury concentration between adults of the two species. Adult seaside sparrows had 2.2 times greater feather mercury compared with adult swamp sparrows ($F_{1,64} = 19.3$, $P < 0.001$; Table 2). We did not detect a difference in feather mercury concentrations between species in hatch-year birds ($F_{1,26} = 0.03$, $P = 0.88$; Table 3). The first primary feather had at least three times greater mercury concentration compared with the outer tail feather for adults of both species (Table 4). Swamp sparrow primary feather mercury concentration (2.05 ± 0.25 ppm) was four times greater than tail feather mercury (0.50 ± 0.06, $t = 6.5$, $df = 14$, $P < 0.001$; Table 4). Seaside sparrow primary feather mercury (3.05 ± 0.74 ppm) concentration was three times greater than tail feather concentration (1.01 ± 0.19, $t = 2.5$, $df = 14$, $P = 0.03$; Table 4).

Table 3 *M. georgiana* and *A. maritimus* outer tail feather mercury ppm (dw) levels in all sampled hatch-years at Woodland Beach State Wildlife Management Area, Delaware, USA, from 2007 to 2008

Mercury ppm (dw)	*M. georgiana*	*A. maritimus*
Minimum	0.54	0.99
Maximum	5.68	2.35
Mean (SE)	1.72 (0.282)	1.65 (0.156)
n	19	9

Table 4 Adult *M. georgiana* and *A. maritimus* first primary (P1) and outer tail feather (R6) mercury levels at Woodland Beach State Wildlife Management Area, Delaware, USA, from 2007 to 2008

Species	P1	R6
M. georgiana		
Minimum	0.57	0.20
Maximum	3.57	1.05
Mean (SE)	2.05 (0.25)[a]	0.50 (0.06)[b]
n	15	15
A. maritimus		
Minimum	0.40	0.46
Maximum	10.70	3.13
Mean (SE)	3.05 (0.74)[a]	1.01 (0.19)[b]
n	15	15

Superscript footnote symbols "a" and "b" indicate values that are significantly different

Discussion

Adult Swamp Sparrow Mercury Concentrations

Adult swamp sparrow feather mercury concentration did not increase with age among the four age classes analyzed. The ability for individuals to bioaccumulate mercury with age is determined by the rate of mercury absorption compared with the rate of mercury depuration (Evers et al. 2005; Rimmer et al. 2005). Negative correlations between feather mercury and age suggest that individuals are depurating mercury at a similar or greater rate of uptake (Fevold et al. 2003). Studies reporting mercury bioaccumulation with age, especially for passerines, are limited in their capacity to define age groups and to sample adults from long term-banded populations. This is the first study that examines bioaccumulation in a tidal marsh passerine species, and the results indicate that mercury concentrations in adult swamp sparrows do not increase with age. We suggest that swamp sparrows are able to eliminate mercury at rates equal to or greater than dietary mercury uptake and that age is not the major determinant of internal mercury concentrations for this species at the Delaware site.

Hatch-Year and Adult Mercury Concentrations

Recently fledged sparrows had greater mercury levels than adults for both species. Arithmetic mean mercury concentration in hatch-year swamp sparrows had an average of four times the level found in adults; similarly, young seaside sparrows had two times the level of adults. Feather mercury in hatch-year birds represents exposure from short-term dietary uptake, whereas feather mercury in adults indicates blood mercury at the time of feather molt (Bearhop et al. 2000) and body burden in accordance with yearly molt (Evers et al. 2005). The flight feathers of hatch-year sparrows are grown synchronically while the bird is a nestling, and nestling feather growth is completed shortly after the bird leaves the nest. Sampling these feathers in hatch-year birds thus provides a mechanism for concurrently monitoring mercury levels near a nesting site. Although the transfer of mercury from mother to egg contributes to levels found in feathers of hatch-year birds (Heinz et al. 2010), embryotoxic levels and maternal transfer rates for salt marsh passerine species remains unknown. We did not detect lower mercury concentrations in adult female *versus* male birds, which we would expect if high hatch-year concentrations were caused largely by maternal transfer (i.e., female birds have higher depuration rates due to maternal transfer).

Compared with our findings, other researchers have reported mercury in feathers to be lower in young birds, particularly in piscivorous species, when adult mercury concentrations are compared with concentrations in juvenile birds. Studies of pelagic fish found that adult osprey (*Pandion haliaetus*) had four times the concentration in primary feathers compared with juvenile birds (Cahill et al. 1998), and adult bald eagles (*Haliaeetus leucocephalus*) had 2.7 times greater mercury levels in the breast or contour feathers than those found in their nestlings (Wood et al. 1996). Selective provisioning by sparrow adults may explain why young have greater mercury concentrations at our study site. Adult bird species commonly feed nestlings the highest-quality food or the most protein-rich source to optimize nestling growth (Gill 2007). It is possible that adults are feeding nestling sparrows invertebrates that have greater mercury concentrations than what they are feeding on themselves, and this could partially explain why nestlings had greater concentrations of mercury than adults. In tidal marsh habitats, invertebrates are abundant during the breeding season and are the main food source for sparrows (Post and Greenlaw 2006). Because food is typically not limited in tidal marshes during the breeding season and sparrows are opportunistic foragers (Post and Greenlaw

2006), selective foraging by adults on lower-quality food items (such as seeds) is not likely. Adults must be feeding nestlings a significantly greater amount of invertebrates at higher trophic levels to make up for the differences we found in this study.

Another possible explanation, in addition to selective provisioning, is that nestlings may ingest mercury at a greater rate than adults because of their accelerated growth rates, which are supported by a high volume of food (for a given body size). The greater mercury concentrations of young birds could be an effect of increased mercury dosage at the nestling stage compared with mercury intake of adults due to the nutritional and metabolic demands of the young. This hypothesis would also explain why hatch-year seaside and swamp sparrows had similar concentrations of mercury because nestlings in general have similar metabolic demands.

Nestling condition is important for both the immediate fledgling and later adult fitness (Gill 2007). The nestling stage, before internal mercury is depurated into growing feathers, has been suggested as the developmental stage with the greatest toxicological exposure (Ackerman et al. 2011), and our data support this hypothesis for tidal marsh sparrows. Furthermore, nestlings are rapidly growing neural networks during this period, and the nestling stage is likely to be the most sensitive stage for producing life-long phenotypic change due to mercury exposure. The fact that these two windows overlap presents tidal marsh passerines with an increased risk for toxicological effect.

Our findings suggest that young sparrows can depurate large amounts of mercury during their first molt. Once the first molt after the juvenal plumage (first prebasic molt) is complete, the internal body burden is lowered, but it has the potential to increase again until the next molt occurs (Condon and Cristol 2009). The concentrations found in adults of our study suggest that the adult body burden does not return to the greater levels that we found in nestlings, presumably because adults are able to eliminate mercury at rates equal to or greater than dietary mercury uptake and are consuming less food on a per-unit body-weight basis compared with nestlings. Our data suggest that dietary intake of mercury is the exposure route for nestling seaside and swamp sparrows. Differences between nestlings and adults most likely reflect variances in mercury intake due to differences in metabolic demands and possibly selective provisioning by adults.

Interspecific Differences in Mercury

We detected differences between species with adult seaside sparrows having two times the mercury concentration in tail feather compared with adult swamp sparrows. Both sparrow species complete their flight feather molt on the breeding grounds before migration (Dwight 1900; Post and Greenlaw 2009). At the Delaware site, adult male swamp sparrows stay on territory into early October, and banded birds caught in mist-nets in late September into October were in heavy molt (R. G. Greenberg, personal observation, October 2008). Mercury concentrations detected in feathers represent internal tissue burdens and exposure at the time of molt.

Differences in mercury exposure rates between sympatric species have been explained by difference in diet or habitat use (Shriver et al. 2006). Species selecting food items at a greater trophic level have greater mercury burdens (Cristol et al. 2008). The differences in mercury levels we found may be explained by foraging behavior if seaside sparrows are ingesting food, such as spiders, which can have greater mercury concentrations compared with other arthropods (Cristol et al. 2008). Although we did not sample prey items, one study reported that spiders were rarely found in the diet of seaside sparrow nestlings based on ligature samples (Post and Greenlaw 2006). Previous research at our Delaware site found that 15 % of the adult swamp sparrows sampled by gastral lavage contained spiders as part of their diet (B. J. Olsen, unpublished data, 2007). Given that seaside sparrows in our study had greater concentrations of mercury than swamp sparrows, seaside sparrows must be ingesting considerably more spiders or other higher trophic level invertebrates compared with swamp sparrows to explain the differences in mercury concentrations we detected.

Interspecific differences we reported can also be explained if methylmercury concentrations vary within microhabitats of the marsh used by the sparrows. For example, one study found saltmarsh (*A. caudacutus*) and seaside sparrows to be opportunistic feeders with adults showing only minor differences in the food they selected (Post and Greenlaw 2006). These minor differences were attributed to the different microhabitats in which the sparrows forage; seaside sparrows made greater use of less dense salt marsh grasses (*S. patens, S. alterniflora, D. spicata*) and shallow ponds, and saltmarsh sparrows made greater use of dense stands of salt marsh grasses (Post and Greenlaw 2006). At our study site, swamp and seaside sparrows nest in close proximity, but they partition the marsh selecting different vegetation as nesting sites (Warner 2009). Seaside sparrows at our site generally nest and forage in salt marsh grasses (*S. alterniflora, S. patens, D spicata*), which are indicative of a more regularly flooded tidal marsh ecosystem (Westervelt et al. 2006), whereas swamp sparrows nest and forage in shrubs and bulrush (*I. frutescens*, *B. halimifolia*, and *S. americanus*), species that represent more upland ecosystems (Westervelt et al. 2006). A study of saltmarsh and Nelson's (*A. nelsoni*) sparrows breeding in the same tidal marsh found blood

mercury levels to be 1.7 times greater in saltmarsh compared with Nelson's sparrows (Shriver et al. 2006); these two species also partition the marsh for nesting sites, and the taxon with the greater mercury load generally inhabits more regularly flooded areas of the marsh. Because methylmercury production is related to several environmental variables (organic carbon, sulfur speciation, and soil properties (O'Driscoll et al. 2011), concentrations can be markedly different within a given site. Differences in methylmercury concentrations within microclimates of the marsh where two species forage could also explain the mercury differences we found between adult seaside and swamp sparrows.

Mercury Variation With Feather Type

Feather mercury levels in adults represent chronic exposure over time, and the stability of mercury in the feather (Appelquist et al. 1984) makes it a good tissue for investigating yearly body burdens (Evers et al. 2005). The primary feathers had at least three times greater mercury levels than the outer tail feathers, a pattern that was shown in both species. In adult sparrows, wing and tail feathers are molted sequentially within feather type, and both feather types molt simultaneously. Thus, the innermost primaries and tail feathers are molted at the same time, and the outermost primaries and tail feathers are molted at the same time. Our two samples reflect feathers molted early (first primary feather) and later (outer tail feather). Feather molt sequence explains the difference in mercury between the two feather types because the feathers molted first should have greater mercury levels than later molted feathers (Altmeyer et al. 1991), a pattern shown in this study and earlier studies of piscivorous and raptorial species (Appelquist et al. 1984; Dauwe et al. 2003; Furness et al. 1986).

Mercury in Tidal Marsh Sparrow Feathers

Mercury in the first feather to molt indicates a consistent record of yearly internal body burdens compared with other feather types (Braune 1987). If that pattern holds true for all avian species, the concentration of mercury in the first primary feather is closely reflective of internal tissue burdens. In our study, sparrows had first primary feather concentrations that averaged 2.05 ppm (dw) $\pm$ 0.25 SE for swamp sparrows and 3.05 $\pm$ 0.73 ppm for seaside sparrows. The availability of published feather mercury data for tidal marsh sparrows is limited; however, a few studies are available for comparison purposes that use the same feather type. Average primary feather mercury concentrations reported for Nelson's sparrows nesting in tidal marshes of North Carolina reported a level of 2.94 ppm (dw) (Winder and Emslie 2011), which is similar to the concentrations found in the primary feathers of seaside sparrows at our site in Delaware. These levels were markedly greater than those of inland Nelson's sparrows wintering in North Dakota and Ontario: 0.98 and 1.21 ppm (dw), respectively (Winder and Emslie 2011). The only other reports of mercury in feathers of tidal marsh sparrows are in two species wintering in Virginia: Nelson's sparrows, which had mean concentrations of 2.08 ppm (dw) $\pm$ 0.40 SE, and saltmarsh sparrows, which had greater concentrations, 5.42 ppm (dw) $\pm$ 0.65 SE (Cristol et al. 2011). This study sampled breast feathers during the wintering season, however, making comparisons with our data difficult. Yet here again, an insectivorous passerine that is more tightly associated with tidal marshes (saltmarsh sparrow) showed an increased risk of methylmercury exposure compared with a species that is less constrained within the ecosystem (Nelson's sparrows).

Conclusion

Swamp and seaside sparrows are tidal salt-marsh endemics, and like all tidal marsh species they are limited in their breeding habitat and continue to face major conservation threats from habitat development, sea level increase, and pollution. The Delaware Bay estuary has potentially high levels of mercury due to heavy atmospheric deposition in the Northeast (Evers et al. 2005), point and nonpoint contamination sources (Mitchell and Gilmour 2008), and environmental characteristics that are conducive to high methylmercury production. This study documents (1) the first reports on feather mercury levels for seaside and swamp sparrows, (2) that swamp sparrows are able to expel mercury at net rates over their lifetime that are equal or greater than mercury intake and therefore are not accumulating mercury with age, (3) nestlings are able to expel large amounts of internal mercury by way of their first feather molt, and (4) exposure to sparrow species is different. Our study supports that the nestling stage, before feather growth, is the period when seaside and swamp sparrows have the greatest exposure to dietary mercury and the largest body burdens. This suggests that although adult sparrows may be able to limit mercury accumulation, exposure is highest during the critical physiological development that occurs in the nest, and adults may thus experience toxicological impacts from earlier developmental stages if concentrations exceed threshold levels.

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