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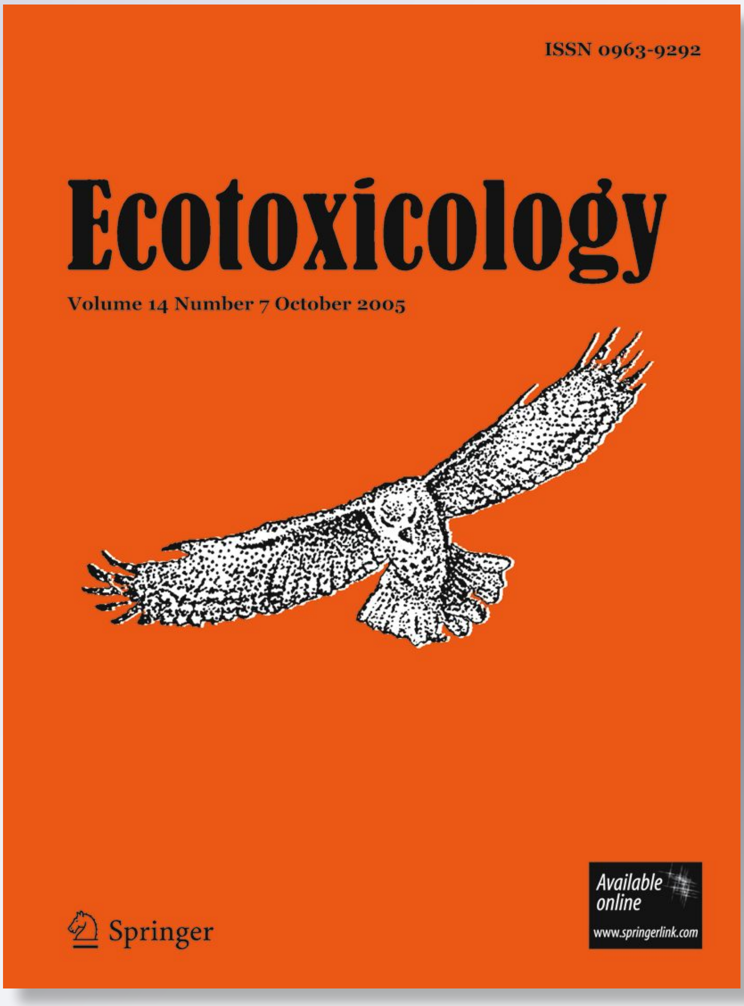

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Abstract Environmental mercury exposure of birds through atmospheric deposition and watershed point-source contamination is an issue of increasing concern globally. The saltmarsh sparrow (*Ammodramus caudacutus*) is of high conservation concern throughout its range and the potential threat of mercury exposure adds to other anthropogenic stressors, including sea level rise. To assess methylmercury exposure we sampled blood of the northern nominal subspecies of saltmarsh sparrows (*A. c. caudacutus*) nesting in 21 tidal marshes throughout most of the species' breeding range. Blood of tree swallows (*Tachycineta bicolor*) was sampled concurrently at three of these sites to provide a comparison with a well-studied songbird that is a model species in ecotoxicology. Arithmetic means (±1 SD) ranged from 0.24 ± 0.06 µg g^{-1} wet weight (ww) in Connecticut to 1.80 ± 0.14 µg g^{-1} ww in Massachusetts, differing significantly among sites. Comparison to tree swallows indicates that mercury exposure is significantly higher in saltmarsh sparrows, making them a more appropriate bioindicator for assessing risk to methylmercury toxicity in tidal marsh ecosystems.

Keywords Saltmarsh sparrow · Tree swallow · Methylmercury · New England · Long Island

Introduction

Environmental mercury (Hg) is a widespread contaminant in the northeastern USA and is transported through atmospheric and local watershed sources (Evers and Clair 2005). A potent neurotoxin, methylmercury (MeHg), even at low concentrations can cause subtle, but permanent damage to the neurological and reproductive systems of wildlife (Wolfe et al. 1998). Studies with piscivorous wildlife (e.g. Common Loon, *Gavia immer*) (Scheuhammer 1987; Burgess and Meyer 2008; Evers et al. 2008) have demonstrated significant impacts from atmospheric Hg deposition on avian reproductive success. Recent studies of Hg exposure in bird species that eat invertebrates also indicate elevated exposure (Cristol et al. 2008; Custer et al.

The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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2007; Edmonds et al. 2010; Evers et al. 2005; Rimmer et al. 2010) and impacts on reproductive success and survival (Brasso and Cristol 2008; Hallinger et al. 2011; Schwarzbach et al. 2006).

Freshwater wetlands generally serve as areas for converting less bioavailable ionic Hg to more bioavailable MeHg (Driscoll et al. 2007), thus making obligate wetland birds especially vulnerable to high levels of Hg contamination (Evers et al. 2005). Rates of total Hg accumulation are higher in wetlands because of the strong association of Hg with organic matter (Grigal 2002) and wetlands often support sulfate-reducing bacteria, which can methylate Hg (Benoit et al. 2003). Few studies have focused on the impact of Hg exposure on invertivorous birds within tidal marshes, but recent evidence suggests that the northern subspecies of saltmarsh sparrows (*Ammodramus caudacutus caudacutus*, SSTS) nesting in New England were exposed to Hg (Lane et al. 2008) and had higher blood Hg levels than the congeneric Nelson's sparrow (*A. nelsoni*) nesting in the same marshes (Shriver et al. 2006).

The saltmarsh sparrow (SSTS) is an obligate salt marsh species of high conservation concern due to multiple threats (i.e. restricted range, sea level rise due to climate change (Nicholls 2004)) and habitat degradation. The threat of Hg exposure adds to this list of anthropogenic stressors. Consequently, the species is classified as globally vulnerable to extinction (IUCN 2009) and is listed as one of the top conservation priorities species (Dettmers and Rosenberg 2000; US Fish and Wildlife Service 2002; Rich et al. 2004). Spending their entire annual cycle in salt marsh habitats, SSTS should be an excellent indicator of the health of the regions' estuaries. The SSTS is comprised of two subspecies including *A. caudacutus caudacutus* (northern form) that breeds along the coast from Maine south to New Jersey and *A. c. diversus* (southern form) that breeds along the coast from New Jersey to Virginia (Greenlaw and Woolfenden 2007). We believe, based on historical range information, all the sparrows sampled were the northern subspecies, the genetic testing in the future will provide a definitive identification of the subspecies.

The objective of this study was to assess Hg exposure of the northern form of SSTS on their breeding range, to determine whether Hg poses an additional threat to this species of high conservation concern. Tree swallows (*Tachycineta bicolor*, *TRES*) have been used as an indicator species in numerous contaminant studies and there is a great deal of contaminant related literature available on the species (Bishop et al. 1995; Custer et al. 1998; Gerrard and St. Louis 2001; St. Louis et al. 1993) as it is a model species for ecotoxicology related investigations (McCarty 2001). Tree swallows have been used specifically for Hg studies in the past (for ex. Brasso and Cristol 2008; Custer et al. 2007). An additional objective of this paper was to compare SSTS with TRES to provide a context for interpreting SSTS blood Hg concentrations.

Methods

Study sites

This study encompassed 21 salt marsh complexes (Fig. 1) with 25 sampling sites: eight estuaries in southeastern Maine (five on Rachel Carson NWR, RCNWR); three in New Hampshire; one estuary with six sampling sites in Massachusetts (four on Parker River NWR); two in Rhode Island; three in Connecticut; and three on Long Island, New York.

Capture and sampling

All capture and blood sampling occurred in the summers of 2004–2008. We used 12-m mist nets with 30–36 mm mesh. Birds were flushed from the vegetation into the nets and banded with a USGS metal band. A beach umbrella was used to shade the birds during handling. We placed 14 nest boxes in Scarborough Marsh, 16 boxes in Furbish Marsh, and 20 boxes in Parker River NWR to attract nesting TRES which could be captured by hand. We determined sex, age (adult or hatch year), and breeding status for each bird. All males sampled had well developed cloacal protuberances and all females had heavily vascularized brood patches indicating breeding condition. Hatch year birds were not used for this study. To expedite the female birds' return to their nests and nestlings, they were sampled and released prior to processing males. Mercury results from adult birds only are reported in this paper. Venipuncture of the cutaneous ulnar vein with a 27-gauge sterile disposable needle allowed collection of 50–70 μl blood into heparinized mylar-wrapped tubes for Hg analysis. The capillary tubes were sealed with Critocaps®, stored in plastic vacutainers on ice for up to 6 h before freezing at −17°C. We released all birds unharmed within 10–20 min of capture. All banding and sampling was conducted under appropriate state and federal permits.

Lab analysis

All analyses were for total Hg, because it has been shown that in songbird blood 95% of total Hg is MeHg (Rimmer et al. 2005). All blood Hg concentrations are expressed in $\mu g\ g^{-1}$ wet weight (ww). All samples were analyzed at Texas A&M University Trace Element Research Laboratory in College Station, Texas using direct combustion/trapping atomic absorption method on a Milestone DMA 80. This approach has been incorporated by the U.S.

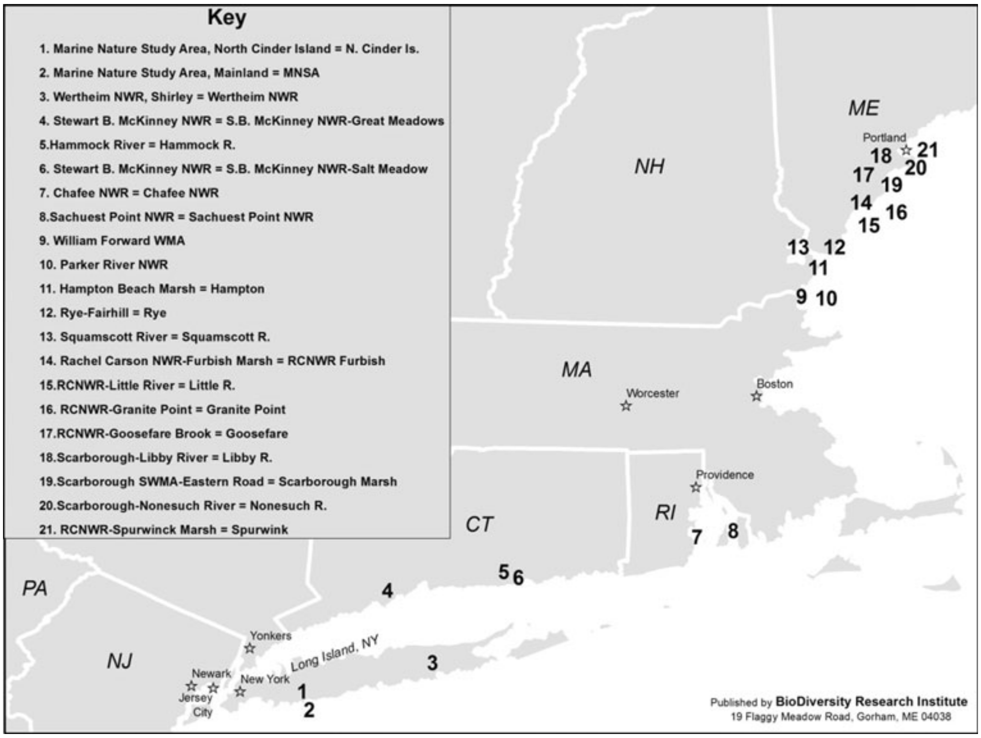

Fig. 1 Saltmarsh sparrow sampling locations (and key to site name abbreviations used in text) in the Northeastern USA, 2004–2008

Environmental Protection Agency (EPA) in EPA SW-846 Method 7473. Calibration utilized a blank and four calibration standards in each of the two detector cells. Instrument response was evaluated immediately following calibration, and thereafter, following every 20 samples and at the end of each analytical run by running two certified reference materials and a check blank. Instrument detection limit was 0.05 ng.

Statistical analyses

To analyze the impact of study site, sex, and year on SSTS blood Hg concentrations, we fit a series of linear mixed-effect models. Initial exploration of the data revealed strong heteroscedasticity, therefore, we log-transformed Hg concentrations for statistical analyses but untransformed data are presented in tables and figures. Only the adult age group was used in analyses. The full model included fixed effects for region (state) and sex, as well as random effects for year, study location within region, and individual bird. State was used to indicate region not because of any assumed relationship with jurisdictional boundaries, but because the study sites within each state were typically close to each other. Thus, state provided a convenient labeling framework to describe regional-scale variability and separate it from local, fine-scale site variability. To assess the statistical significance of fixed effects, we followed Faraway (2006) in fitting the full model and reduced models by maximum likelihood, where the reduced models were formulated by leaving out each fixed effect in turn. If the resulting likelihood ratio test was close to 0.05, we bootstrapped the likelihood ratio statistic to produce a more accurate test (Faraway 2006). We performed similar tests to evaluate whether the random effects had variances significantly greater than zero; however, the random effects included in the model almost certainly have nonzero variance in reality, so we interpreted the results of these tests cautiously. Residual plots and quantile–quantile plots were used to evaluate approximate conformance of the data to model assumptions of normality. After the final model was selected, we used restricted maximum likelihood to obtain more efficient parameter estimates (Faraway 2006).

To examine the impacts of site and year on TRES blood Hg, we fit models similar to those for SSTS. Again, we log-transformed blood Hg. Because TRES Hg was collected at only three sites, we omitted region from the analysis, and modeled location as a fixed effect. The full model included site and sex as fixed effects, with a random effect of year. Finally, to compare SSTS and TRES, we combined the data from SSTS that were collected at the same sites as TRES were collected with the TRES data, and fit a full model that included species, site, and sex as fixed effects, with a random effect of year. Hypothesis testing proceeded as described above for SSTS. All statistical analyses used the lme4 library (Bates and Maechler 2009) of the R statistical package (R Development Core Team 2009).

Results

Saltmarsh sparrows

We collected blood samples for Hg analysis from 653 adult (285 female and 368 male) SSTS from 25 sites in 21 estuaries across the breeding range of this subspecies. We found the lowest mean Hg concentrations in the sparrows from Hammock River in Connecticut (0.24 μg g^{-1}) and the highest in Parker River NWR in Massachusetts (1.80 μg g^{-1}) (Appendix).

There was strong regional variation in Hg concentration (as defined by state boundaries). Region was a highly significant factor in the model (Likelihood ratio test, $\chi^2 = 22.5$, df = 5, $p = 0.0004$), with Massachusetts showing the highest concentrations, followed by New York, Rhode Island, Maine, New Hampshire, and Connecticut (Appendix). There was a tendency for males to have lower Hg than females, but this was not quite statistically significant (Bootstrap percentile test, $p = 0.0541$; difference in log Hg of −0.0195, or approximately 4% of average Hg). Although this parameter was not statistically significant in a strict sense, we followed the rationale of Altman (1991), Hurlbert and Lombardi (2009), and others in not pedantically excluding a term from the model over a trivially small difference in p-value. We further note that inclusion of sex in the model slightly reduced the Akaike information criterion (ΔAIC = 1.74), offering some support for its inclusion. Location ($p < 0.0001$) and year ($p = 0.0039$) were significant random effects in the model, while individual bird was not ($p = 0.1561$). However, the lack of significance of individual was almost certainly due to the limited number of recaptures in the data set and hence a lack of statistical power. In the final model fit by restricted maximum likelihood, residual variability after the effects of region and sex were removed was explained by site within region (standard deviation = 0.163, 63% of variance), individual bird (standard deviation = 0.050, 6% of variance), year (standard deviation = 0.037, 3% of variance), and individual measurement residual (standard deviation = 0.110, 28% of variance). Hence, even within study region, specific site was an important determinant of blood Hg in SSTS (Fig. 2, Appendix). Blood Hg levels from Parker River NWR were consistently (in 2004–2008) and significantly higher than sparrows from all other sites ($p < 0.05$; Fig. 2).

Saltmarsh sparrow versus tree swallows

We sampled 77 adult (66 female and 11 male) TRES over a 3 year period. We only used adult SSTS data from the same sites and years as TRES for this analysis. Blood Hg in TRES followed a similar pattern to that for SSTS, with location being highly significant and the sites in Maine having lower Hg than those in Massachusetts (Likelihood ratio test, $\chi^2 = 25.025$, df = 3, $p < 0.0001$). Male TRES had statistically significant lower blood Hg than females (bootstrap test, $p = 0.0416$. Year was a statistically

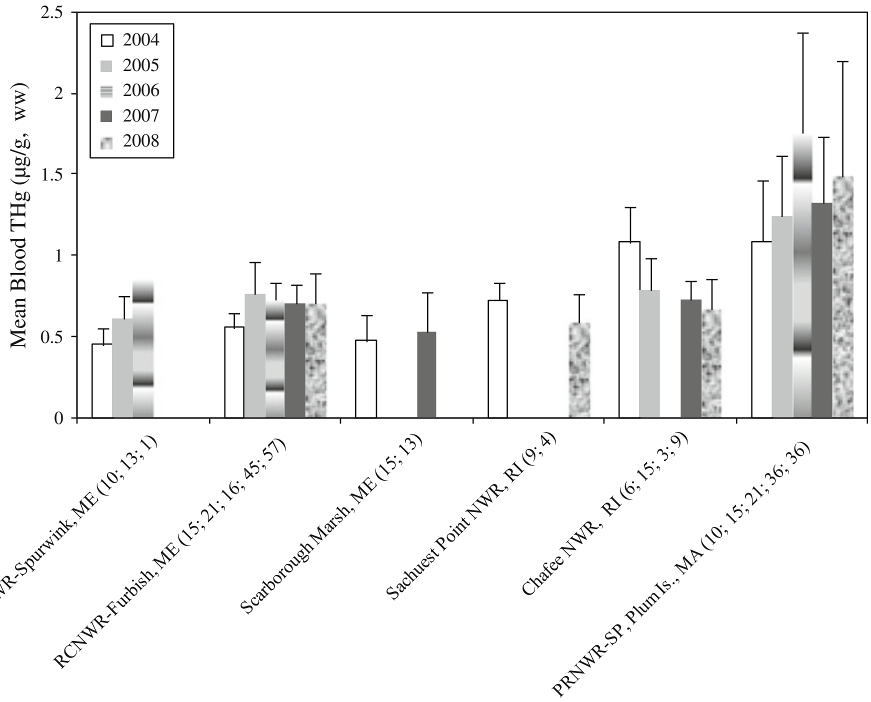

Fig. 2 Arithmetic mean (±1 SD) of whole blood THg levels in adult saltmarsh sparrows from sites sampled in multiple years, 2004–2008. *RCNWR* Rachel Carson National Wildlife Refuge, *PRNWR-SP* Parker River National Wildlife Refuge-Salt Pannes, (*n*) number of birds sampled

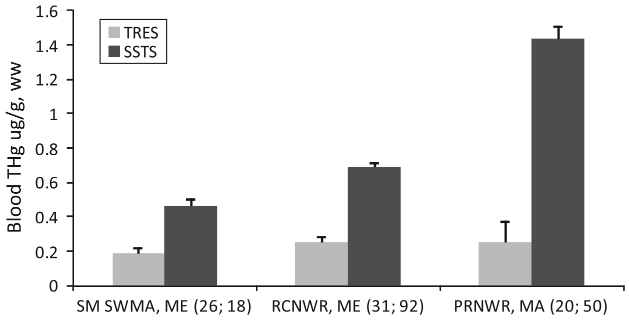

Fig. 3 Arithmetic mean (±1 SD) of whole blood Hg concentrations (μg g^{-1}, ww) in adult tree swallows (TRES) and adult saltmarsh sparrows (SSTS), 2004–2005, 2008. *RCNWR* Rachel Carson National Wildlife Refuge, *SM SWMA* Scarborough Marsh State Wildlife Management Area, *PRNWR* Parker River National Wildlife Refuge

significant random effect (bootstrap test, $p = 0.0439$). Finally, the statistical model comparing SSTS and TRES blood Hg showed similar results to those for the analysis of TRES alone, with site and sex giving statistically significant fixed effects and a statistically significant random effect of year. Species was highly significant in this model ($p < 0.0001$), with TRES showing an average difference of −0.5227 in log Hg, or approximately 30% lower blood Hg on average than SSTS (Fig. 3, Table 1).

Discussion

Saltmarsh sparrow-geographic blood Hg variation (among site differences)

Blood total Hg in SSTS varied along the New England/New York coast. This is likely a result of differences in Hg input and/or methylation within salt marsh systems, or sparrows at different marshes or sites in the same marsh may select different prey items. Amphipods, spiders, flies, grasshoppers and moths are an important component of nestling SSTS diets in southern Maine, in New Hampshire (K. O'Brien and T.P. Hodgman, personal observation) and on Long Island, New York (Post and Greenlaw 2006; Merriam 1979). Diet is the demonstrated route of Hg exposure in wildlife. Amphipods and spiders are likely a source of Hg exposure in SSTS and variation in the abundance or contamination level of these prey groups could explain variation in avian blood Hg among sites. George and Batzer (2008) reported that amphipods contained much higher concentrations of Hg than other organisms found higher on a wetland food chain, such as Odonates and crayfish. Cristol et al. (2008) found spiders from Hg-contaminated sites had the highest MeHg concentrations of all invertebrates collected from three species of songbirds. Further study of diet and Hg level in specific prey items is necessary.

Blood Hg concentrations in SSTS varied by site and by region. Even among multiple sites within a single estuary, such as Plum Island in MA, we found significant effects of site on Hg concentrations in the blood of SSTS. For example, the Green Belt sparrows had significantly lower blood Hg levels than the Salt Panne site.

One likely reason for elevated Hg levels in sparrows from the Parker River NWR compared to the other sites on the New England coast is because sampling efforts were concentrated in the salt marsh situated between the Merrimack and Parker Rivers. Both rivers likely carry Hg-polluted waters from interior watersheds to the coast (Evers et al. 2007). The Merrimack River, flowing through some of the most urbanized and industrialized areas of southern New Hampshire and northeastern Massachusetts, has been identified as a biological Hg hotspot for the region (Evers et al. 2007).

Interspecies-blood Hg comparisons

Difference between saltmarsh sparrows and tree swallows

The significant difference in blood Hg levels between the species (Table 1) is likely a reflection of their foraging habits and diet. We speculate that prey items in TRES diets contain less Hg than the prey consumed by SSTS. Saltmarsh sparrows forage exclusively in the salt marsh (Greenlaw and Rising 1994, Merriam 1979) and a large

Table 1 Geometric mean blood Hg concentrations, sample size (*n*) and CIs (μg g^{-1}, wet weight) for adult tree swallows and adult saltmarsh sparrows in Maine and Massachusetts 2004, 2005, 2008

Site	Tree swallow			Saltmarsh sparrow		
	Mean	*n*	95% CI	Mean	*n*	95% CI
Scarborough Marsh, ME	0.16	26	0.12–0.20	0.43	18	0.35–0.54
RC NWR-Furbish Marsh, ME	0.24	31	0.21–0.27	0.67	93	0.63–0.70
Parker River NWR, MA	0.25	20	0.22–0.28	1.32	50	0.57–3.0

proportion of their diet likely consists of benthic invertebrates. Saltmarsh sparrows forage on the ground for Diptera, Hemiptera, Homoptera, Araneida, and amphipods (Merriam 1979, Post and Greenlaw 2006), or from the vegetative layer on moths and grasshoppers (Post and Greenlaw 2006).

Exposure of TRES to Hg may have been less than to SSTS because they may feed on a different group of aquatic insects or insects that drifted in and which may have originated outside of the salt marsh (Blancher and McNicol 1991, Quinney and Ankney 1985). Tree swallow diets included adult Diptera, Odonata, Ephemeroptera (mayflies), and a variety of small terrestrial prey such as leafhoppers (Homoptera) (Quinney and Ankney 1985) and spiders represented a small (<4%) proportion of diet (Mengelkoch et al. 2004). In addition, there may be a significant difference in Hg levels between larval and adult stages of many insects. Larval guts contain significant fraction of Hg in the organism (Elwood et al. 1976) and when most insects and other invertebrates emerge, they molt their final exoskeleton, which includes the gut lining and the guts, leaving a significant portion of the Hg behind (Hildebrand et al. 1980 and Sarica et al. 2005).

Based on the evidence presented in this paper, we believe that a proportion of SSTS population is likely exposed to harmful levels of Hg on their breeding grounds in the Northeast United States. There is limited published information available on Hg effect levels in songbirds. Edmonds et al. (2010) reports 1.0 µg/g (ww) Hg in blood as a level of concern for rusty blackbird (*Euphagus carolinus*) based on common grackle (*Quiscalus quiscula*) egg injection experiment described in Heinz et al. 2009. Brasso and Cristol (2008) report minimal reproductive success effects at adult blood Hg levels of 2–4 µg/g. Due to a lack of published data on sparrow LOAEL, we speculate it is likely to be between 1 and 4 µg/g. In our conservative approach we use 1.0 µg/g (ww) as LOAEL estimate at this time. Of all 653 adult SSTS sampled for this study 179 or 27% had blood Hg above 1.0 µg/g.

Conservation implications

As a bird of conservation concern, it is critical that biologists investigate the potential parameters affecting the reproductive success of SSTS and find other biomarkers of contaminant exposure and effects. Mercury could affect physiology and behavior, and ultimately the reproductive success, of the species breeding in high Hg salt marshes. Gjerdrum et al. (2005) and Shriver et al. (2007) examined nest-site selection and nesting success in the species and found that successful nests were initiated in time to avoid flooding from spring tides. Mercury, as a neurotoxin that is known to disrupt neurochemical signaling pathways in mammals (Basu et al. 2005a, b), could potentially alter SSTS's normal behavior, including nest-site selection and/or nest synchronization with the tide cycles, thereby making them more vulnerable to flooding. Temperature change, rising water levels, or changes in wetting–drying regimes and sulfide production could all potentially increase Hg bioavailability in coastal habitats.

Based on the results of 5 years of sampling we conclude that SSTS have elevated blood Hg levels across the sampling sites in their breeding range and are at potential risk for reduced reproductive output at several sites. We believe *Ammodramus* species: Nelson's sparrow (*A. nelsonii*) (Winder and Emslie 2011) in northeastern Maine and Canada, SSTS from mid coast Maine south to New Jersey and seaside sparrow (*A. maritimus*) in Delaware and south (Warner et al. 2010) make an appropriate bioindicator species in tidal marsh ecosystems. A suitable bioindicator should be easy to sample to address management issues, be widespread in the habitat in question, and occupy that habitat exclusively (Golden and Rattner 2003). The three sparrow species fit the above criteria for the tidal marsh ecosystems. In addition, TRES sampled in tidal marshes have significantly lower blood Hg concentrations than SSTS (Fig. 3) and thus this commonly used and suitable bioindicator in freshwater habitats may not be the most appropriate species to use for Hg monitoring in tidal marshes.

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Appendix

See Table 2.

Table 2 Breeding saltmarsh sparrow arithmetic mean of whole blood total Hg concentrations and range (μg g^{-1}, ww) from New England and New York salt marshes, 2004–2008 (sites are arranged in geographic order from north to south, *RCNWR* Rachel Carson National Wildlife Refuge, *MNSA* Marine Nature Study Area, *n* number of birds sampled)

Site	Year	Mean ± std	Range	*n*
Maine				
Scarborough Marsh	2004	0.47 ± 0.16	0.23–0.82	15
	2007	0.52 ± 0.24	0.22–1.10	14
Scarborough-Libby R.	2005	0.31 ± 0.06	0.27–0.42	7
Scarborough-Nonesuch R.	2005	0.45 ± 0.09	0.39–0.52	2
RCNWR				
Spurwink	2004	0.45 ± 0.10	0.26–0.60	10
	2005	0.61 ± 0.14	0.40–0.61	13
	2006	0.85	–	1
Granite Point	2004	0.55 ± 0.11	0.46–0.66	3
Goosefare	2004	0.50 ± 0.12	0.32–0.75	13
Little R.	2004	0.74 ± 0.08	0.64–0.84	7
Furbish	2004	0.56 ± 0.09	0.33–0.69	15
	2005	0.76 ± 0.21	0.47–1.44	21
	2006	0.73 ± 0.11	0.58–0.95	16
	2007	0.71 ± 0.12	0.53–1.00	45
	2008	0.70 ± 0.19	0.39–1.40	57
New Hampshire				
Rye	2008	0.81 ± 0.13	0.57–1.00	30
Squamscott R.	2008	1.10 ± 0.23	0.64–1.70	32
Hampton	2008	0.32 ± 0.15	0.14–0.93	33
Massachusetts				
Parker River NWR				
Salt Pannes	2004	1.10 ± 0.38	0.67–1.70	10
	2005	1.24 ± 0.38	0.81–2.20	15
	2006	1.80 ± 0.61	1.00–3.70	21
	2007	1.30 ± 0.42	0.52–2.20	36
	2008	1.50 ± 0.70	0.62–3.30	35
Area A	2006	1.65 ± 0.14	1.50–1.90	8
Area B	2007	1.20 ± 0.30	0.65–1.40	5
Lot 2	2007	1.20 ± 0.40	0.58–2.20	13
Plum Island-Essex Co. Green Belt	2006	0.88 ± 0.15	0.62–1.20	14
William Forward WMA	2007	1.53 ± 0.21	1.40–1.80	3
Rhode Island				
Ninigret NWR Complex				
Sachuest Point	2004	0.72 ± 0.11	0.54–0.87	9
	2008	0.59 ± 0.18	0.38–0.82	4
Chafee	2004	1.10 ± 0.22	0.86–1.40	6
	2005	0.79 ± 0.19	0.41–1.20	15
	2007	0.87 ± 0.17	0.51–1.20	31
	2008	0.67 ± 0.15	0.38–0.95	16
Connecticut				
S.B. McKinney NWR				
Great Meadows	2004	0.54 ± 0.11	0.39–0.73	15
	2005	0.61 ± 0.14	0.44–0.96	10
Salt Meadow				
Hammock R.	2004	0.24 ± 0.06	0.18–0.34	6
Long Island, New York				
Wertheim NWR	2007	0.83 ± 0.19	0.52–1.20	26
MNSA	2008	0.68 ± 0.26	0.45–1.10	5
N. Cinder Is.	2008	1.50 ± 0.32	0.93–1.90	13

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