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Long-term monitoring of mercury in adult saltmarsh sparrows breeding in Maine, Massachusetts and New York, USA 2000–2017

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Abstract

Here we report on the results of a long-term study of mercury exposure in a songbird species, the saltmarsh sparrow (*Ammodramus caudacutus*). We measured total mercury concentrations in blood ($n = 840$) and feathers ($n = 560$) of adult saltmarsh sparrows at six locations between 2000 and 2017: Rachel Carson National Wildlife Refuge (RCNWR) in Wells, Maine; Scarborough Marsh State Wildlife Management Area in Scarborough, Maine; Parker River National Wildlife Refuge on Plum Island, Massachusetts; Pine Neck Preserve in Southampton, Long Island, New York; and North Cinder and North Green Sedge Islands off the coast of Long Island, New York. During the 12–17 year sampling periods, we found that mercury exposure differed by site and year but there was no consistent temporal trend across sites. Blood mercury concentrations declined only at RCNWR in Maine. We also found seasonal variation in blood mercury concentrations and a positive relationship between mercury concentrations of blood and innermost primary feather, but not between blood and tail feather.

Keywords Tidal marshes · Mercury · Saltmarsh sparrows · Long-term monitoring

Introduction

Mercury (Hg) is a global and widespread contaminant in the Northeastern United States and is transported through atmospheric and local watershed sources (Evers and Clair 2005). However, Hg deposition is not uniform across the region (Evers and Clair 2005) where coastal sites receive more Hg in precipitation than inland locations (Vanarsdale et al. 2005). Hg is ubiquitous (Evers and Clair 2005) and common in wetlands (Grigal 2002). Estuaries and associated tidal marshes offer water resources, vegetative cover, and nursery habitat for a multitude of species that depend on them for survival during one or more parts of their life cycle and many of which are in decline (Correll et al. 2017; Greenberg et al. 2006). These ecosystems and habitats are under increasing pressure from the growing human population and the associated stressors such as eutrophication, erosion, pollution, and sea level rise that result from human activities (US EPA 2004). At the turn of this century, approximately 90% of the coastal waters on the East Coast were under fish consumption advisories for Hg and other persistent pollutants (US EPA 2004).

Methylmercury (MeHg), the organic form of Hg, is a potent neurotoxin; even at low, environmentally relevant concentrations, permanent damage to the neurological and reproductive systems of wildlife can occur (Whitney and Cristol 2017; Scoville and Lane 2013; Wolfe et al. 1998). Studies with piscivorous birds (e.g., common loon, *Gavia immer*; Burgess and Meyer 2008, Evers et al. 2008, Scheuhammer et al. 2007) have demonstrated significant effects of MeHg on avian reproductive success. Recent studies of Hg exposure in songbird species that consume invertebrates also indicate potential risk (Cristol et al 2008; Custer et al. 2007; Evers et al. 2005; Lane et al. 2011; Rimmer et al. 2010; Winder 2012). Impacts on

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reproductive success, health, and survival have also been reported (Brasso and Cristol 2008; Hallinger et al. 2011; Jackson et al. 2011; Schwarzbach et al. 2006; Whitney and Cristol 2017).

Few studies have focused on the impact of MeHg exposure on invertivorous birds within tidal marshes, but recent evidence suggests that the saltmarsh and seaside sparrows (*Ammodramus caudacutus and A. maritimus*) breeding in the northeastern United States are exposed to elevated and potentially harmful levels of this chemical (Lane et al. 2011, Warner et al. 2010). The saltmarsh sparrow is an obligate salt marsh species of high conservation concern due to multiple threats (i.e., restricted range, the threat of sea level rise upon tidal marsh habitats (Nicholls 2004), and other types of habitat loss or degradation such as housing, industrial development, and other land use conversion). Consequently, this species is classified as globally endangered by BirdLife International (2017); is a species of highest concern by Partners in Flight (Rosenberg et al. 2016); and is one of the top conservation priorities species (US Fish and Wildlife Service 2008). Recent trends report a 5–9% annual decline in population since 1990 (Shriver et al. 2015; Correll et al. 2017) and population simulations in response to sea level rise suggest possible extinction of the species as soon as 2035 (Field et al. 2017).

Birds that consume prey species high on the wetland food chain (e.g., spiders) can be exposed to high concentrations of MeHg (Cristol et al. 2008), and thus be indicative of Hg exposure at many lower trophic levels in the ecosystem. The saltmarsh sparrow is an appropriate indicator of tidal wetlands Hg because it is an obligate salt marsh species, spends its entire life cycle in the salt marshes of the tidal Atlantic coast, and feeds strictly on invertebrates during the summer breeding season. This study focused on multiyear trends in saltmarsh sparrow Hg exposure across tidal marshes in the Northeastern United States because conservation concern for saltmarsh sparrows is high and the potential for Hg to have negative effects on this species could complicate efforts to conserve and restore populations. Our goal with this study was to follow up on previous work describing spatial patterns of Hg exposure in saltmarsh sparrows (Lane et al. 2011; Shriver et al. 2006) and to understand how Hg exposure in this species is changing over time in northeastern tidal marshes. Spatiotemporal trends in Hg exposure could be useful for determining specific sites or populations that are at greatest persistent risk and require additional research or management support.

Furthermore, we define which nonlethally collected tissues are most useful for describing trends in Hg exposure and how sampling strategies influence estimates of annual Hg trends.

Methods

Study area

This study encompassed six salt marsh complexes in three states in the Northeastern United States. All sites were selected based on existing data and presence of breeding saltmarsh sparrows. The New England region includes marshes in Maine and Massachusetts; the New York region includes the Long Island sites. Two sites were located in southeastern Maine: Scarborough Marsh State Wildlife Management Area (Scarborough Marsh) in Scarborough and the Rachel Carson National Wildlife Refuge (hereafter Rachel Carson NWR) in Wells. One salt marsh was located in northeastern Massachusetts: Parker River National Wildlife Refuge (hereafter Parker River NWR). Three sites were located on Long Island, New York: Pine Neck Preserve in Southampton, and two islands in Hempstead off the coast of Long Island: North Cinder and North Green Sedge (Fig. 1). Not every site was sampled annually (Table 1).

Capture and sampling

All capture and blood sampling occurred during summers June–August of 2000–2001 and 2004–2017.

To capture birds, we placed two to six 12 m mist nets, with 30–36 mm mesh, in a semicircle or wall in the tidal marsh habitat. Birds were flushed from the vegetation into the nets and banded with a USGS metal band. All adult birds were in breeding condition (females had a well-developed brood patch and males had enlarged cloacal protuberance). We determined sex and age (adult or hatching year by plumage characteristics) for each bird under shaded conditions to reduce heat stress to birds. The capture protocol varied for males and females: females were processed first to expedite their return to nests or nestlings. Blood samples were collected from all birds captured that had evidence of breeding (i.e., enlarged cloacal protuberance or presence of a brood patch) via venipuncture of the cutaneous ulnar vein with a 27-gauge sterile disposable needle allowing collection of 50–70 ug blood into heparinized mylar-wrapped tubes. The capillary tubes were sealed with Critocaps®, stored in plastic vacutainers on ice for up to 6 h before freezing at −17 degrees Celsius. We pulled two outer tail feathers (sixth rectrices, R6) and an innermost primary (P1) feather from all adults. We placed feathers in a paper coin envelope, labeled and refrigerated until analysis. We released all birds unharmed within 20 min of capture. Hatch-year birds were not used in this study because of the low rate of capture. All banding and sampling was conducted nonlethally under required state and federal permits.

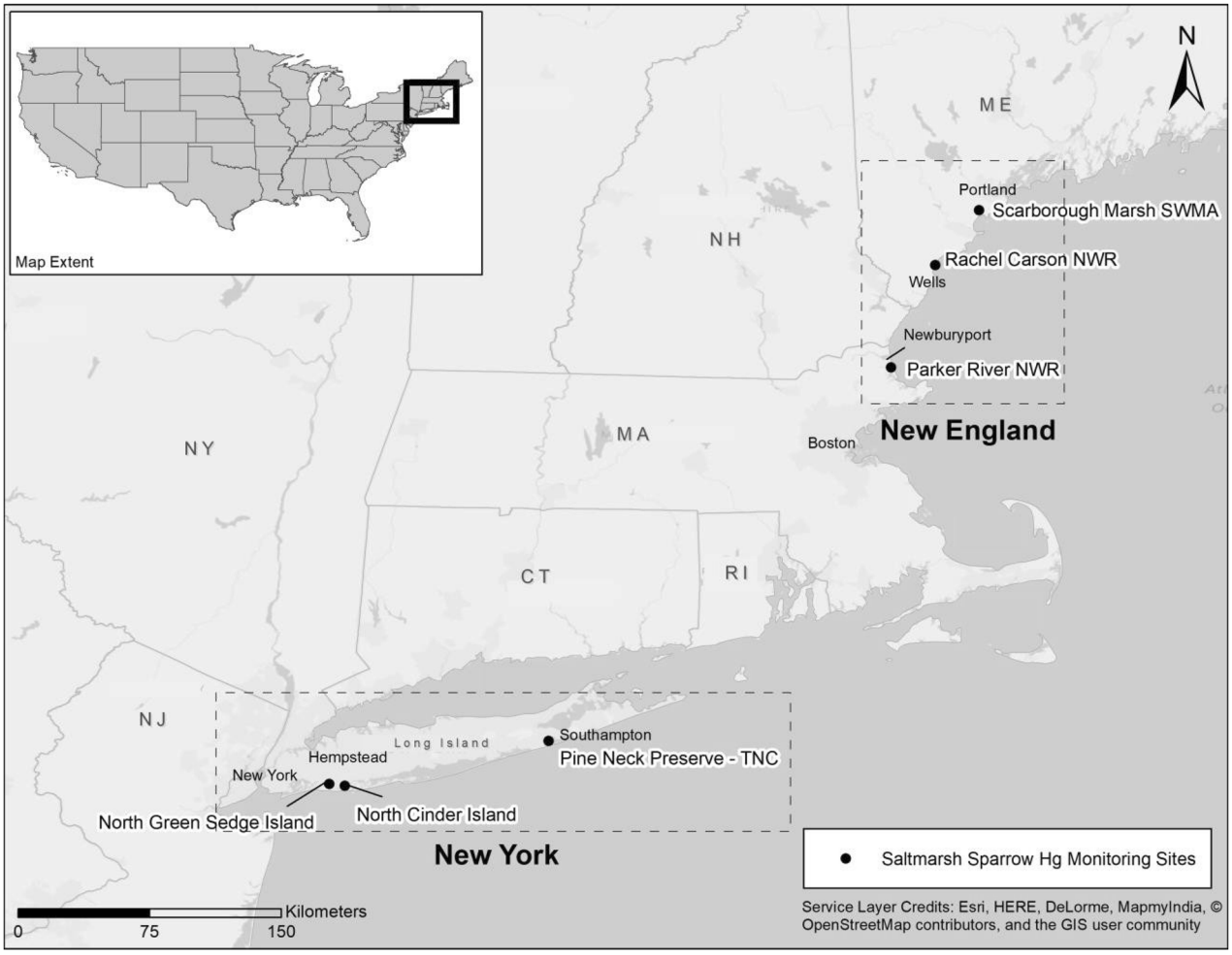

Fig. 1 Map of the sampling locations of saltmarsh sparrows in Maine, Massachusetts, and New York

Table 1 Sites and years of saltmarsh sparrow sampling during 2000–2017 in the northeast of the United States

State/Region site years sampled		
Maine/New England	Rachel Carson NWR	Annually 2004–2015
Maine/New England	Scarborough Marsh SWMA	2000, 2001, 2004, 2007–2009
Massachusetts/New England	Parker River NWR	Annually 2004–2016
New York/Long Island	North Cinder Island	2008, and 2010–2017
New York/Long Island	North Green Sedge Island	2011–2014, 2016–2017
New York/Long Island	Pine Neck Preserve, TNC	2010, 2011, 2013–2015

Lab analysis

All songbird blood and feathers were analyzed for total Hg at Texas A&M University Trace Element Research Laboratory (TERL) in College Station, Texas and the Wildlife Toxicology Lab (WTL) at Biodiversity Research Institute (BRI) in Portland, Maine. We measured cost effective total Hg instead of MeHg because ~90–95% of total Hg is MeHg in songbird blood (Edmonds et al. 2010, Rimmer et al. 2005) and over 80% in feathers (Bond and Diamond 2009; Thompson and Furness 1989). MeHg in feathers is stable and sequestered for long time periods (Applequist et al. 1984). All samples were analyzed using a thermal decomposition and atomic absorption spectrophotometry technique with a direct Hg analyzer (DMA 80, Milestone Incorporated) using US EPA Method 7473. For analysis, whole blood was expressed from the capillary tube into a nickel or quartz receptacle while feathers were cut in half and placed into nickel receptacles. 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. Quality control methods, including analyzing one of the DOLTs certified reference materials (DOLT 4, DOLT 5) and BCR 463 or CE 464 were used at both labs to ensure consistent analytical precision and accuracy. Percent recoveries of certified reference materials were >90% and relative percent difference (RPD) of duplicates were within 10%. All samples exceeded the method detection limit (0.001 mg/kg). All blood and feather Hg concentrations are expressed in μg/g wet weight (ww) and dry weight (dw), respectively.

Statistical analysis

To assess whether saltmarsh sparrow Hg concentrations are changing across tidal marsh sampling locations and years we used a general linear modeling framework. In this framework we use blood Hg concentrations as the response variable, and sampling site, year, day of capture, and sex as explanatory covariates. As Hg exposure data are typically right skewed, we log-transformed the data to better approximate a normal distribution and achieve better model fit. Year was analyzed as a categorical variable to allow for nonlinear relationships over time and to best account for sites not being sampled across all years. Day of year was analyzed as a continuous ordinal date. Birds sampled after August were removed from the analysis to avoid confusion from using potentially migrating birds with Hg exposure from other sites.

As sampling protocols were different between the New York tidal marsh sites and the New England sites (Maine and Massachusetts), we fit two different models for each group. New York sites were each visited on one day in early July every year of the study, so we tested a model that accounted for sex, site, and year but did not account for variation in Hg exposure throughout the summer. Specifically, the model we built tested the interaction between site and year while accounting for sex. The New England sites were sampled repeatedly across the summer breeding season, thus we tested a model that included site, year, sex, and day of the year as base terms along with an interaction between site and year and the square of day of the year, which allowed Hg concentration to vary nonlinearly across the breeding season. While a model that encompassed all sites would have provided greater statistical power, we were unable to use that approach because we sampled the New York region only once per year and nesting started a month earlier than in New England (Greenlaw et al. 2018). Only one model was tested for each set of sites as our interest was in the variation in Hg across year and site and goodness of fit for both models was assessed using R^2 and visual assessments of residuals. Recaptures of individuals in the same year were not included in this analysis, but recaptures between years were included. Despite some correlation in individuals among years (see below for additional analysis) we viewed an interannual recapture as an independent assessment of site Hg availability. Importance of individual model terms was assessed using an F-test and significance of model parameters was assessed using a t-test. For comparative purposes, we combined the predictions of annual Hg exposure levels (with 95% confidence intervals around each prediction) together to show the similarities and differences in temporal trends across all sites. Post hoc Tukey tests were used to evaluate the pairwise differences among years within each site, and the sites within a region. This analysis is intended to confirm patterns in the models and explore the relative spatial and temporal variation in the data.

Hg concentrations in the blood were compared to Hg concentrations in the innermost primaries and the tail using a Pearson correlation coefficient via paired sampling events within the same individuals. A t-test was used to assess the significance of the correlation. A general linear model with blood Hg as the response and feather Hg as the independent variable was used to fit lines to describe the direction of the effect for each comparison.

Finally, we explored trends in Hg exposure for individuals recaptured and sampled in multiple years (i.e., bioaccumulation). To conduct this analysis, we applied a general linear mixed model that used an individual as a random effect to describe saltmarsh sparrow blood Hg concentrations. Effectively, this allowed us to conduct a repeated measures style analysis that controlled for variation in Hg levels across individuals.

We calculated the number of years since initial capture to estimate interannual bioaccumulation and used this as a categorical variable to account for nonlinear relationships over time. We built two models using sex, site, and number of years since capture to describe changes in log-transformed blood Hg for the two sites with large numbers of recaptures at Parker River NWR and Rachel Carson NWR. The first model tested for all the base effects and the second tested the interaction of site and year to assess site-specific bioaccumulation. If the interaction is statistically significant then that model will be selected, otherwise the base model will be selected. Model fit was assessed similarly to the general linear models though R^2 was decomposed into marginal (fixed effects only) and conditional (fixed and random effects) versions. Importance of model terms was assessed using an F-test and the Kenward–Rogers estimate of residual degrees of freedom, and individual parameters were assessed using profile likelihood estimates of 95% confidence interval.

Statistical analyses and figures were all conducted and created in R (R Core Team 2017), package "lme4" (Bates

Table 2 Summary of total mercury (THg) concentrations (μg/g, ww) in blood of female and male saltmarsh sparrows for six sites in the northeastern United States, all sites in ME and MA are in New England and all sites in NY are on Long Island New York

Site	Years sampled	Female THg mean ± SD (μg/g, ww)	*N* Female	Male THg mean ± SD (μg/g, ww)	*N* Male
Parker River NWR-MA	2004–2016	1.58 ± 0.73	184	1.27 ± 0.51	137
Scarborough Marsh-ME	2000–01; 2004, 2007–2009	0.600 ± 0.32	30	0.562 ± 0.18	62
Rachel Carson NWR, Wells-ME	2004–2015	0.720 ± 0.19	100	0.642 ± 0.16	121
Pine Neck Preserve-Long Island, NY	2010–11; 2013–2015	0.990 ± 0.31	27	1.12 ± 0.20	17
North Cinder Is.-Long Island, NY	2008; 2010–2017	1.55 ± 0.40	50	1.48 ± 0.31	54
North Green Sedge Is.-Long Island, NY	2011–14; 2016–2017	1.06 ± 0.22	21	1.07 ± 0.27	37

Table 3 Summary of total mercury (THg) concentrations (μg/g, dw) in feathers of adult saltmarsh sparrows for six sites in the northeastern United States, all sites in ME and MA are in New England and all sites in NY are on Long Island New York

	First primary (P1)		Sixth tail (R6)	
Site	THg mean ± SD	N P1	THg mean ± SD	N R6
Parker River NWR-MA	13.9 ± 10.3	40	2.0 ± 1.4	84
Scarborough Marsh-ME			1.9 ± 1.8	11
Rachel Carson NWR, Wells-ME	5.9 ± 4.2	35	2.3 ± 2.3	87
Pine Neck Preserve-Long Island, NY	12.2 ± 7.8	44	1.8 ± 0.75	33
North Cinder Is.-Long Island, NY	13.1 ± 10.8	61	2.4 ± 2.2	85
North Green Sedge Is.-Long Island, NY	15.7 ± 9.0	36	2.0 ± 1.1	45

et al. 2015), package "piecewiseSEM" (Lefcheck 2015), and the package "ggplot2" (Wickham 2009).

Results

Across six sites, we analyzed 840 blood samples, 216 innermost primary feather samples, and 345 tail feather samples (Tables 2 and 3). Not all sites were sampled each year, but each site had at least five samples taken each year over five or more years.

Mercury exposure differences in age, site, and year

Both the New York and New England general linear models had strong goodness of fit. The overall R^2 was 0.59 for New York and 0.58 for New England, and both regions showed good evidence of strong fit from residuals to predicted value plots and quantile–quantile plots of model predicted values and the original data.

In the New York model, sex did not predict Hg levels (F-test, $F_{1,204} = 0.65$, $p = 0.65$) and the site/year interaction was marginal in importance (F-test, $F_{9,196} = 1.9$, $p = 0.05$). There were large differences among sites (F-test, $F_{2,203} = 89.7$, $p < 0.001$) and years (F-test, $F_{8,196} = 14.7$, $p = <0.001$), with site differences explaining more variation than temporal differences. Using post hoc Tukey tests, we found more variation in Hg exposure among sites than within sites. By comparing sites in years in which multiple sites were sampled, we found that North Cinder Island was higher than North Green Sedge Island in 2011, 2016, and 2017; North Cinder Island was higher than Pine Neck in 2011, 2013, 2014, and 2015; and North Green Sedge was higher than Pine Neck in 2013 and lower in 2015 (Table 4). Variation within sites was more limited (Table 5). Within North Cinder Island, we found 2010 was significantly lower than all other years and 2013 was lower than 2011 and 2015. North Green Sedge had one significant difference among year combinations, 2011 was higher than 2014. Pine Neck had more variation with 2010 and 2011 both being lower than 2013 and 2014, and 2015 being higher than 2014.

Overall, the majority of years showed site differences but only a few years within sites showed significant differences. The integration of these differences demonstrates differing patterns among sites: North Green Sedge shows a slight decline in Hg levels with large amounts of confidence interval overlap among years; North Cinder Island shows a strong sinusoidal pattern across years; and Pine Neck shows a decline in Hg levels in the middle sampling years then returns to the beginning values in the last year sampled (Fig. 2).

In the New England model, we found a significant effect of both sex (F-test, $F_{1,625} = 15.2$, $p < 0.001$) and nonlinear day of the year (F-test, $F_{1,625} = 22.6$, $p < 0.001$). Females had higher blood Hg concentrations than males ($\beta = -0.1$,

Table 4 List of significantly different sites within paired sampling year using a post hoc Tukey significance test

Year	Site1	Site2	Difference	SE	*t* value	*p* value
2010	Pine Neck	North Cinder	−0.44	0.07	−5.86	0.000
2011	North Cinder	Green Sedge	0.41	0.10	3.94	0.016
	Pine Neck	North Cinder	−0.39	0.11	−3.44	0.075
2013	Pine Neck	Green Sedge	−0.34	0.10	−3.46	0.074
	Pine Neck	North Cinder	−0.46	0.10	−4.75	0.001
2014	North Cinder	Green Sedge	0.45	0.09	4.91	0.000
	Pine Neck	North Cinder	−0.72	0.10	−7.14	0.000
2015	Pine Neck	North Cinder	−0.49	0.10	−4.87	0.000
2016	North Cinder	Green Sedge	0.27	0.07	3.79	0.027
2017	North Cinder	Green Sedge	0.42	0.09	4.74	0.001
2004	Parker River	Rachael Carson	0.61	0.15	4.15	0.010
	Scarborough	Parker River	−0.85	0.15	−5.76	0.000
2005	Parker River	Rachael Carson	0.50	0.12	4.12	0.012
2006	Parker River	Rachael Carson	0.85	0.12	7.07	0.000
2007	Parker River	Rachael Carson	0.55	0.07	7.32	0.000
	Scarborough	Parker River	−0.91	0.11	−8.03	0.000
2008	Parker River	Rachael Carson	0.65	0.08	8.17	0.000
	Scarborough	Parker River	−0.85	0.13	−6.76	0.000
2009	Parker River	Rachael Carson	0.78	0.10	7.80	0.000
	Scarborough	Parker River	−0.79	0.10	−7.80	0.000
2010	Parker River	Rachael Carson	0.66	0.12	5.44	0.000
2012	Parker River	Rachael Carson	1.19	0.22	5.45	0.000
2013	Parker River	Rachael Carson	1.11	0.18	6.16	0.000
2014	Parker River	Rachael Carson	0.78	0.15	5.36	0.000

All pairwise combinations were tested. A *p* value of 0.000 is <0.0005. Alpha is set to 0.1 for inclusion on this list. All differences with higher *p* values are not included

$p = 0.001$) and we found there was a peak in blood Hg levels around mid-July (approximately ordinal day 200, Fig. 3). After controlling for these effects, we found that the site/year interaction was highly significant (F-test, $F_{1,625} = 3.0$, $p < 0.001$) as well as the base effects for site (F-test, $F_{2,624} = 344.5$, $p < 0.001$) and year (F-test, $F_{13,613} = 10.9$, $p < 0.001$). Using a Tukey post hoc test we found that again there were more significant differences across sites than within sites, although there was a significant trend detected in Parker River (Table 4). In comparing sites among multiple years, we found that Parker River was higher than Rachel Carson NWR from 2004–2014 and higher than Scarborough Marsh in 2004 and 2007–2009; Scarborough Marsh and Rachel Carson NWR were similar in all years.

When comparing years within sites we find that Parker River NWR is the site with the most changes, many differences were found between the high years (2006, 2009, and 2012) and lower years (2011 and 2014). The only years with differences at Scarborough Marsh were 2004 and 2009 and Rachel Carson NWR had a low year in 2014 that was significantly different from 2005–2010 (Table 5). The result of these effects shows a nonlinear decrease in Hg at Rachel Carson starting in 2013, sinusoidal patterns of yearly variation at Parker River, and no consistent significant pattern at Scarborough Marsh (Fig. 2).

Mercury exposure across tissue type

From most birds, we collected multiple tissues, which included blood, outer tail feathers, and innermost (first) primaries. Blood was collected from all animals and is the reference for these tissue comparisons; paired blood and first primary feathers were collected from 213 individuals, and blood and tail feathers were collected from 336 birds. We found that blood and primary feather Hg levels had a positive Pearson correlation of 0.44 (95% CI: [0.32, 0.54]) while blood and tail Hg had no correlation at −0.04 (95% CI: [−0.15, 0.07]; Fig. 4).

Individual changes in mercury exposure

The general linear mixed effect model used to describe changes in individual Hg levels over time also had strong

Table 5 List of significantly different years within each sampling site using a post hoc Tukey significance test

Site	Year 1	Year 2	Difference	SE	*t* value	*p* value
North Cinder	2010	2008	0.28	0.08	3.61	0.046
	2012	2010	−0.32	0.09	−3.50	0.064
	2013	2010	−0.44	0.09	−5.13	0.000
	2014	2010	−0.34	0.09	−3.84	0.023
	2016	2010	−0.35	0.07	−4.92	0.000
	2017	2010	−0.28	0.07	−3.80	0.025
	2013	2011	−0.51	0.11	−4.74	0.001
	2015	2013	0.34	0.09	3.58	0.051
Pine Neck	2013	2010	−0.46	0.09	−5.34	0.000
	2014	2010	−0.62	0.09	−6.90	0.000
	2013	2011	−0.46	0.11	−4.26	0.004
	2014	2011	−0.62	0.11	−5.59	0.000
	2015	2014	0.47	0.11	4.40	0.003
Green Sedge	2014	2011	−0.33	0.09	−3.75	0.030
Scarborough	2009	2004	0.47	0.12	3.97	0.020
Rachael Carson	2014	2005	−0.67	0.15	−4.45	0.003
	2014	2006	−0.61	0.16	−3.89	0.029
	2014	2007	−0.60	0.14	−4.33	0.005
	2014	2008	−0.57	0.14	−4.20	0.008
	2014	2009	−0.63	0.15	−4.27	0.007
	2014	2010	−0.60	0.17	−3.63	0.066
Parker River	2012	2004	0.51	0.13	3.94	0.023
	2011	2006	−0.44	0.10	−4.38	0.005
	2014	2006	−0.68	0.11	−6.43	0.000
	2012	2007	0.37	0.08	4.49	0.003
	2014	2007	−0.37	0.09	−4.25	0.007
	2014	2008	−0.44	0.09	−4.69	0.001
	2011	2009	−0.39	0.09	−4.17	0.009
	2014	2009	−0.63	0.10	−6.39	0.000
	2014	2010	−0.48	0.09	−5.14	0.000
	2012	2011	0.50	0.09	5.63	0.000

All pairwise combinations were tested. A *p*value of 0.000 is <0.0005. Alpha is set to 0.1 for inclusion on this list. All differences with higher *p*values are not included

goodness of fit with an overall conditional R^2 of 0.62 (0.42 marginal R^2) with no relationship between residuals and fitted values and a highly linear quantile–quantile plot. The random effect of individual was a significant proportion of the total model variance (0.044 variance in that model term and 0.086 residual) and had a strong effect on predictive capacity of the model (0.2 R^2 increase). In the fixed effects, sex was not important (Kenward–Rogers adjusted F-test, $F_{1,85.5} = 0.47$, $p = 0.5$) and there was large variation among sites similar to what we saw in the models for all captures (Kenward–Rogers adjusted F-test, $F_{4,80.7} = 20.9$, $p < 0.001$). The number of years since capture was not important (Kenward–Rogers adjusted F-test, $F_{4,134.9} = 0.25$, $p = 0.24$), showing a slight, nonsignificant decline (Fig. 5). Tests for an interaction between site and year were not statistically significant and there was no evidence of site-specific patterns of bioaccumulation in these individuals.

Discussion

Hg concentrations in saltmarsh sparrows breeding in the northeastern United Stated varied by sites, time of year, and among years and by tissue type. The results of this study indicated: (1) there was significant variance within sites, and temporal patterns of Hg exposure varied significantly by site with some sites decreasing and others fluctuating around a stable mean; (2) we identified a seasonal pattern of Hg peaking in mid-late July for New England saltmarsh sparrows; (3) we found that blood and first primary Hg concentrations had a positive Pearson correlation of 0.44

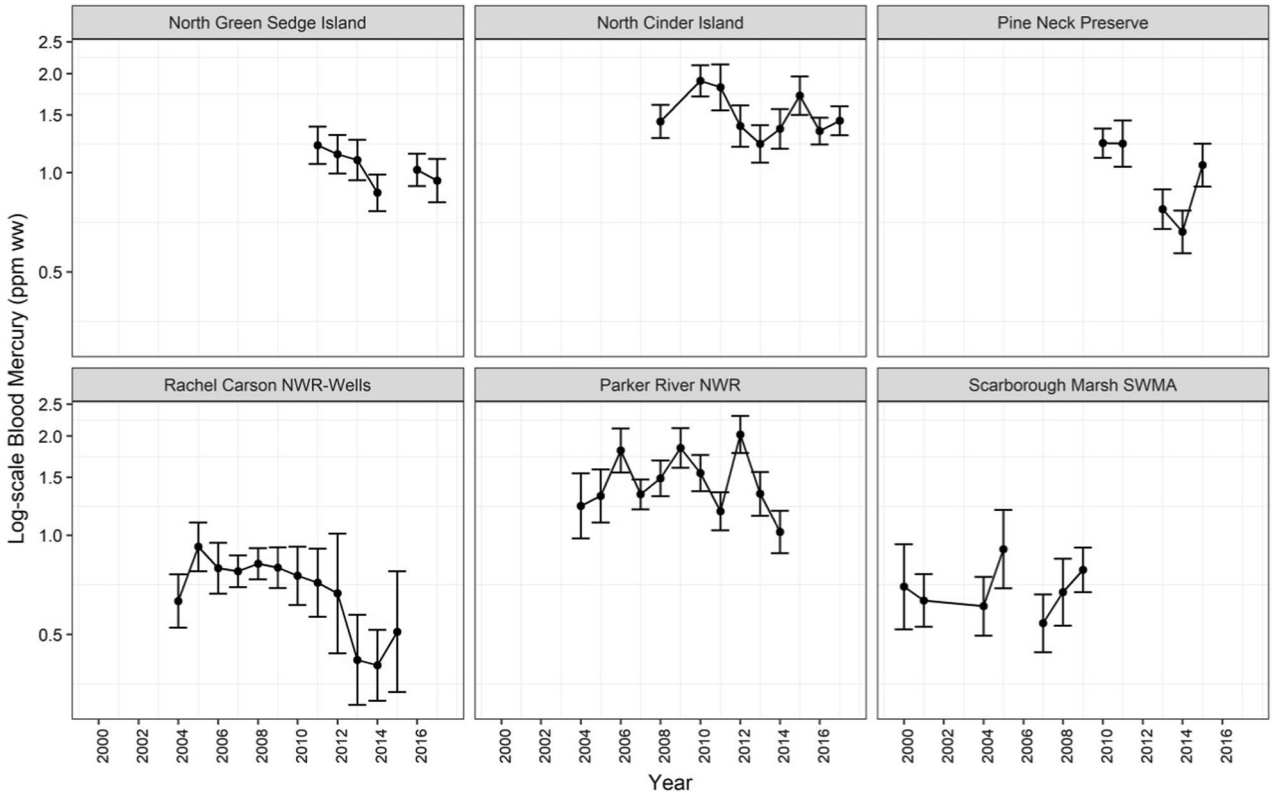

Fig. 2 The expected values of annual blood mercury exposure (μg/g, ww) by site from two general linear models (the New York model, top, and the New England model, bottom). Estimates of annual mercury concentrations control for variance in mercury across date in the New England sites and sex in all sites. The *y*-axis is on a log scale to make comparisons among sites more clear and error bars represent 95% confidence intervals of the predictions

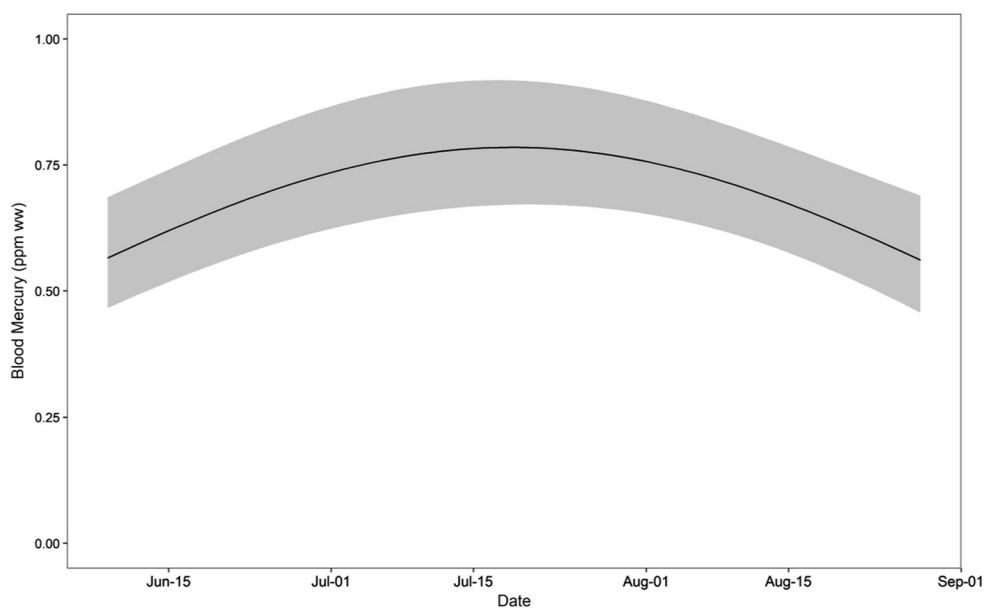

Fig. 3 Model predicted values of blood mercury (μg/g, ww) in saltmarsh sparrows as a function of date. The shaded area represents the 95% confidence interval of the estimate

(95% CI: [0.32, 0.54]) while blood and tail feather Hg had no relationship with a Pearson correlation of −0.04 (95% CI: [−0.15, 0.07]) Fig. 4; and (4) we found evidence that annual fluctuations in blood Hg on the breeding sites were not associated with multiyear bioaccumulation by aging individuals, as individuals showed no consistent increase in blood Hg exposure between their first capture and later years. Thus, we reason that exposure risk to Hg for

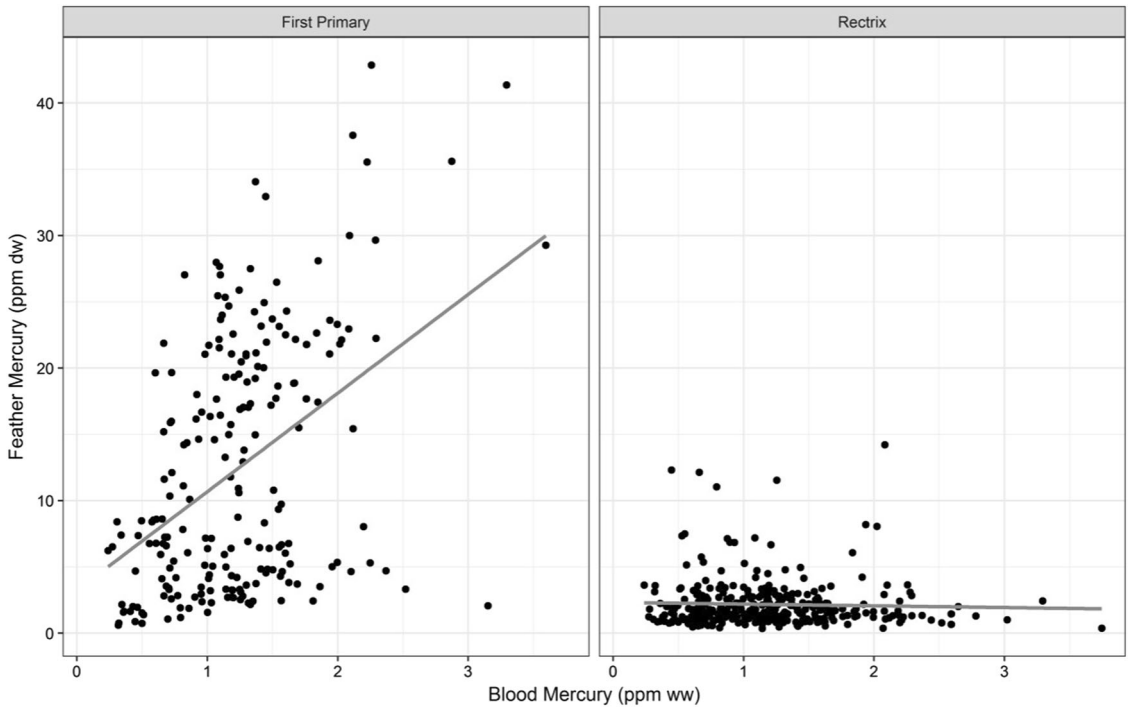

Fig. 4 Scatterplot of the relationship between blood mercury (ppm ww) and mercury in two types of feathers (innermost (first primary) and outer tail feathers, ppm dw) sampled from the same individuals. The gray lines represent the slope coefficient of a linear model between the two variables. The correlation between blood and primary is 0.44, blood and tail feathers is −0.04

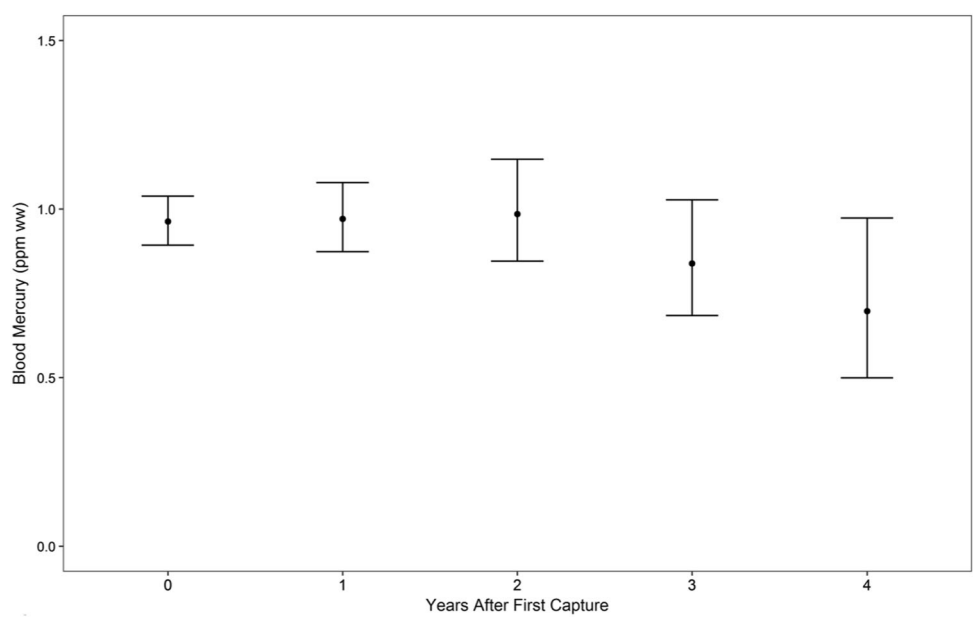

Fig. 5 Expected values of blood mercury (ppm ww) in saltmarsh sparrows across years since first capture based on a repeated measures linear mixed model. Error bars represent 95% confidence interval of the estimate

saltmarsh sparrows breeding in tidal marshes fluctuates with annual variation in ecological and hydrological processes that control the methylation of Hg and the local availability of MeHg. We did not identify strong temporal trends in Hg exposure in this species across the 12–17 year sampling periods, despite increased attention to regulation of mercury discharge into the environment in recent decades.

Changes in mercury exposure over time and space

Blood Hg concentrations varied significantly among sites and years. Sites were often different from each other in the same sampling years with North Cinder Island and Parker River NWR showing consistently high exposure levels. Consistent patterns within sites were difficult to detect because not all sites were sampled in all years; some sites

showed declines, but most appear to be fluctuating around a mean exposure level. There was no evidence that blood Hg concentrations were consistently increasing at any site. The reasons behind these annual fluctuations are currently unclear, but they are not due to sampling biases associated with Hg changing over calendar date or between sexes, as these factors were held constant in our modeling analysis. Thus, annual changes in Hg available to be methylated, methylation/demethylation rates, and availability of MeHg are the most likely causes to variation in annual Hg exposure.

We did, however, detect intra-annual variation in blood Hg concentrations. We found that blood Hg in saltmarsh sparrows increased with residence time on the marsh similar to findings reported in other studies of birds nesting in tidal marshes (Kopec et al. 2018) and other wetlands (Wolfe et al. 2018). Kopec et al. (2018) found similar patterns of seasonal increase during nesting season in blood Hg concentration of Nelson's sparrows (*Ammodramus nelsoni*) and red-winged blackbird (*Agelaius phoeniceus*) reflecting Hg concentrations in the invertebrate diet. Wolfe et al. (2018) found blood THg concentrations in red-winged blackbirds were higher in summer than in spring. There are several possible reasons why blood Hg concentrations change throughout the breeding season. The birds might be shifting their choice of food items—earlier in the summer they may eat prey that are lower on the food web, then in mid-summer they may eat prey that are higher on the food web (such as spiders). Post and Greenlaw (2006) found that the diet of nestling saltmarsh sparrows on Long Island, New York paralleled the prey availability in the marsh. Changes in rainfall and temperature can affect availability of aquatic emergent prey with higher or lower Hg concentrations.

While there is year-to-year variance in saltmarsh sparrow Hg concentrations across all sites, Rachel Carson NWR appears to be showing declines in Hg in recent years. Hg deposition across the United States has declined over the past decade and specifically within the northeastern states (Zhou et al. 2016, Risch et al. 2017), but there is no consistent decline across the six sites that could be expected if regional Hg deposition was driving tidal marsh exposure. Despite this reduction in deposition, we have seen Hg increases in various biota throughout the same time period (Zananski et al. 2011, Stenhouse et al. 2018). Local sources are likely the drivers of Hg exposure at some of these sites, due to nearby industrial activities. For example, a municipal garbage incinerator built in 1989 in the Hempstead area on Long Island likely burned Hg-containing waste, such as thermostats, until the Mercury Thermostat Collection Act of 2013 was signed into law in New York. Correlations with Hg deposition or input into ecosystems and Hg exposure to vertebrates can be complicated to quantify due to temporal lags associated with remobilization of previously deposited Hg via rainfall, organismal movements, or sediment disturbance (Wang et al. 2004). The processes affecting Hg methylation in the salt marshes are complex and information is lacking from New England and New York regions. A synthesis of all available avian Hg data collected from the west coast of the USA found that birds in salt marsh habitats had higher Hg exposure than birds in terrestrial and freshwater habitats (Ackerman et al. 2016). Differences among marsh sites could be due to variation in Hg methylation that is related to local weather conditions, soils, or biota. Marvin-DiPasquale et al. (2002) reported, for example, that microbial MeHg production is highest in the surface sediments collected in the salt marshes vs. open water sites in California, and salinity is reported to have an inverse relationship with methylation rates in anaerobic salt marsh sediments where high salinity sediments appeared to promote demethylation of Hg (Compeau and Bartha 1987). We will consider measuring salinity and pH in future studies. Daily rise and fall of water levels in tidal marshes in addition to flooding can also cause mobilization or remobilization of Hg and increased methylation rates, so habitats with irregular flooding patterns could be local hotspots of MeHg availability (Singer et al. 2016).

Mercury exposure across tissue type

We found a strong correlation between blood and innermost primary feather Hg concentrations, but not with tail feather Hg concentrations. Blood represents recent Hg intake (Monteiro and Furness 2001) and feathers reflect blood Hg levels at the time of feather growth (Bearhop et al. 2000). Feather Hg levels represent both site-specific dietary uptake of MeHg and body burden at the time of feather growth (Burger 1993). This attribute makes feathers a relevant tissue for evaluating chronic body burdens, particularly when considering the stability of MeHg in the feathers (Applequist et al. 1984). Primary feathers in adult saltmarsh sparrows molt or get replaced once a year, at the end of the breeding season, typically in late August/September and the tail feathers are molted twice a year, once after the breeding season in early fall and molt again on the wintering grounds (Pyle 1997). While comparing Hg across feather tracts is difficult, the considerably lower concentrations in tail feathers suggest that there is lower Hg exposure on the wintering grounds of North America (Greenlaw and Woolfendon 2007). Lower blood Hg has been reported on the wintering grounds for this species (Cristol et al. 2011; Winder and Emslie 2012) than on the breeding grounds in the northeast and mid-Atlantic regions (Lane et al. 2011; Warner et al. 2010). Thus, the correlation we found between wing feathers and blood is a reflection of site fidelity between the previous breeding season's molt period and the blood sampled during the current season. Tail feathers

reflect Hg obtained in winter and would not be expected to correlate with Hg concentration from the breeding site. We found that on average Hg concentrations in the innermost primary feathers were seven times higher than Hg in outer tail feathers (Table 3). Warner et al. (2012) found the innermost primary feathers of an obligate salt marsh species, the seaside sparrow, had at least three times greater Hg concentrations than the outer tail feather. Despite primaries being grown almost a year before the blood sample is collected, the innermost primary feathers appear to be useful indicators of Hg exposure in this study system. While feather sampling is less invasive than blood sampling, sources of Hg variation in both tissue types must be considered when interpreting the results. Primary feathers are not always ideal feathers to collect, especially during migration and from long-distance migrants; therefore caution must be used in feather collection. Blood is best used to assess recent exposure to Hg, first primary feather is best when investigating the influence of "breeding site" on any year and tail Hg reflects winter Hg exposure or exposure at a breeding site in hatch-year birds.

Conclusions

Hg appears to be one of many threats that northeastern tidal marshes and associated wildlife populations face. We found 62% of all saltmarsh sparrows sampled in Parker River NWR and 57% of birds sampled on Long Island exceeded 1.2 ppm ww Hg in blood over the course of the study period. Based on the only study of Hg effects on the reproductive success in wild songbird population, the adult Carolina wrens (*Thryothorus ludovianus*) with blood Hg concentration of 1.2 ppm experienced an estimated 20% reduction in nest success (Jackson et al. 2011). Juvenile birds exposed to elevated Hg levels could also face potential neurological damage (Scoville and Lane 2013). Integrating Hg monitoring with other tidal marsh research and monitoring efforts could help us determine if Hg is correlated with behavioral or demographic effects in wild living populations.

Our study found that while there were significant changes in annual Hg exposure to a tidal marsh specialist in the northeast, these variations were not consistent across marshes or years, even after variables like time of year and sex were accounted for. These results suggest that Hg exposure patterns are significantly different across tidal marshes—even marshes that are geographically proximate. Local Hg availability and ecological conditions at each tidal marsh are likely the driving factors for both spatial and temporal variance in blood Hg concentrations in saltmarsh sparrows. We were unable to detect region-wide temporal trends in saltmarsh sparrow Hg exposure, suggesting that regional conditions have not changed over the past 12–17 years, or that saltmarsh sparrows are not a sensitive bioindicator of such changes. Environmental factors such as temperature, rainfall, and tidal flooding could affect Hg availability at each of these sites and more research is needed to quantify these effects.

Standardization of Hg monitoring efforts in tidal marshes is recommended as the means to address questions about determinants of interannual variation in Hg exposure. Blood Hg is a useful endpoint for understanding Hg bioavailability on the breeding grounds in this species as the Hg exposure has occurred in a known and recent timeframe, but if a less invasive method of Hg sampling is required and information on recent exposure is not needed, then primary feathers could be used. Blood sampling should occur during peak Hg exposure in saltmarsh sparrows (i.e., mid-July), while first primary feathers can be sampled anytime during the breeding season before the onset of molt in August. Currently, the saltmarsh sparrow is a critically declining species in North America (Rosenberg et al. 2016). Based on our findings and using Jackson et al. (2011) as an estimate of effect Hg concentrations, these birds could be at risk to Hg effects. Monitoring and assessing the prevalence and effects of Hg in tidal marshes will be important to making successful conservation decisions about this and other species and the limited habitat upon which they rely.

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Compliance with ethical standards

Conflict of interest The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the US Fish and Wildlife Service. The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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