

# Marsh Wrens As Bioindicators of Mercury in Wetlands of Great Salt Lake: Do Blood and Feathers Reflect Site-Specific Exposure Risk to Bird Reproduction?

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**S** Supporting Information

**ABSTRACT:** Nonlethal sampling of bird blood and feathers are among the more common ways of estimating the risk of mercury exposure to songbird reproduction. The implicit assumption is that mercury concentrations in blood or feathers of individuals captured in a given area are correlated with mercury concentrations in eggs from the same area. Yet, this assumption is rarely tested. We evaluated mercury concentrations in blood, feathers, and eggs of marsh wrens in wetlands of Great Salt Lake, Utah, and, at two spatial scales, specifically tested the assumption that mercury concentrations in blood and feather samples from birds captured in a defined area were predictive of mercury concentrations in eggs collected in the same area. Mercury concentrations in blood were not correlated with mercury concentrations in eggs collected within the same wetland unit, and were poorly correlated with mercury concentrations in eggs collected at the smaller home range spatial scale of analysis. Moreover, mercury exposure risk, as estimated via tissue concentrations, differed among wetland units depending upon whether blood or egg mercury concentrations were sampled. Mercury concentrations in feathers also were uncorrelated with mercury concentrations in eggs, and were poorly correlated with mercury concentrations in blood. These results demonstrate the potential for contrasting management actions that may be implemented based solely on the specific avian tissue that is sampled, and highlight the importance of developing avian tissues as biomonitoring tools for assessing local risk of mercury exposure to bird reproduction.



U.S. Geological Survey

## INTRODUCTION

Mercury is a global pollutant that is highly toxic to wildlife, adversely impacting behavior, survival, and reproduction.<sup>1</sup> Continued mercury deposition over time has led to increased efforts and expanded monitoring programs to assess exposure risk to wildlife.<sup>2,3</sup> Piscivorous waterbirds are frequently used as bioindicators of mercury exposure because they occupy a high trophic level and thus are subject to mercury biomagnification,<sup>4,5</sup> and because legacy mercury pollution can cause aquatic ecosystems to have elevated levels of mercury exposure.<sup>6,7</sup>

More recently, insectivorous songbirds have been used to monitor mercury exposure within aquatic and terrestrial food webs<sup>8,9</sup> and some studies have found evidence of reduced reproductive success associated with elevated tissue mercury levels.<sup>10</sup> Insectivorous songbirds are widespread and abundant, occupy both aquatic and terrestrial habitats, have small feeding territories,<sup>11</sup> and often can be sampled easily—all attributes that make songbirds potentially useful as spatially precise bioindicators of mercury exposure to wildlife. However, as with

all potential bioindicators, correct interpretation of results requires an understanding of the species ecology, and how mercury concentrations in different tissues translate to exposure risk.

In particular, it is necessary to select appropriate tissues when using songbirds as bioindicators of mercury exposure. Whole eggs are an ideal tissue for evaluating the risk of mercury exposure in birds because reproduction is among the most sensitive end points for mercury toxicity in birds,<sup>1</sup> eggs have among the best developed toxicity thresholds,<sup>12</sup> and eggs typically represent mercury exposure from a discrete time period during breeding.<sup>13</sup> Therefore, egg mercury concentrations often are the most direct approximation of mercury risk to reproduction in birds. Yet, nonlethal sampling of bird blood and feathers are among the more popular methods for

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approximating songbird mercury exposure in the environment.<sup>14,15</sup> The implicit assumption, however, is that mercury concentrations in blood and feathers are reliable indices of the potential risk of mercury to bird reproduction in the sampled area. Previous studies have found strong positive correlations between a female's blood mercury concentration and mercury concentration in eggs from her clutch,<sup>16,17</sup> but see<sup>18</sup> However, the assumption that mercury concentrations in blood samples of birds captured in a specific area are correlated with mercury concentrations of eggs collected from the same area is rarely tested. Because maternal blood mercury concentrations are correlated with egg mercury concentrations, it often is assumed that mercury concentrations of blood sampled from birds captured in a given area will be reflective of egg mercury concentrations in that same area. Yet, there are several reasons why this might not occur, including bird movements, diet, timing of sampling, sampling of transient individuals, and dynamics of mercury depuration into eggs.

In this paper, we assessed the strength of correlations in mercury concentrations among blood, feathers, and eggs of marsh wrens (*Cistothorus palustris*) breeding in wetlands of Great Salt Lake. In particular, we tested the assumption that blood and feather mercury concentrations of individuals captured in a defined area are reflective of mercury concentrations in eggs collected from the same area. We tested whether mercury concentrations in blood and feathers were correlated with mercury concentrations in eggs at two spatial scales: (1) within the same wetland unit (the scale at which conservation and management actions often occur), and (2) within a smaller area more closely representative of a marsh wren's home range (a scale which relates directly to the species' ecology). We also evaluated individual-based correlations in mercury concentrations among blood and feather samples, and illustrate how the strength of these correlations varies among wetland units and age–sex classes. Our results highlight the potential pitfalls of using proxy tissue matrices, such as bird blood or feathers, without first developing these tissues as suitable biomonitoring tools to infer local risk of mercury exposure to bird reproduction.

## MATERIALS AND METHODS

**Study Area and Sample Collections.** We studied mercury concentrations in marsh wrens at the Bear River Migratory Bird Refuge (BRMBR), Great Salt Lake, Utah (41.47° N, 112.26° W) in June and July of 2011 (see Supporting Information (SI) for study site and species details). We focused on marsh wrens in this study because they have small territories (60–10 000 m<sup>2</sup>), are widespread and abundant,<sup>19</sup> and recent studies have suggested that songbirds in general,<sup>12</sup> and wrens in particular,<sup>10</sup> may be vulnerable to mercury contamination. We captured marsh wrens using mist nets (12 m long, 30 mm mesh) at 12 sites within eight wetland units of the BRMBR. We selected capture sites based on the presence of marsh wrens and active marsh wren nests, but also on proximity to the Bear River and Great Salt Lake. Five sites in four wetland units were located in the region where the Bear River first empties into the refuge complex, and seven sites in four wetland units were located several kilometers away toward the Great Salt Lake, thereby allowing for a potential contrast in mercury concentrations between tissues collected at the Bear River inflow into the BRMBR and outflow from the BRMBR into the Great Salt Lake. Capture sites, both within and across wetland units, were separated by a minimum of 250 m, and most capture sites were

separated by more than 500 m. At each capture site, we erected 1–2 mist nets and broadcast a recording of marsh wren calls and song using a portable audio player and 3-W speaker system. Universal Transverse Mercator (UTM) coordinates were recorded at the midpoint of each mist net using a hand-held GPS unit (position accuracy <5 m). Each captured bird was banded with a U.S. Geological Survey metal band. We used plumage to assign birds to one of two age classes following Pyle:<sup>20</sup> (1) after-hatch-year adults (individuals that hatched in a previous year), and (2) hatch-year juveniles (individuals that hatched in the current year). We identified adult birds' sex, using the degree of cloacal protuberance for males and the presence of a brood patch for females,<sup>20</sup> and we confirmed sex using genetic analysis (Zoogen Services, Davis, CA, USA) for all but two individuals. Unlike eastern marsh wrens, western marsh wrens are thought to undergo a single prebasic molt after the breeding season.<sup>21</sup> However, a few specimens collected in California in the spring were found to be molting some feather tracts.<sup>21</sup> We therefore sampled multiple feather tracts to account for differences in feather mercury concentrations associated with potential differences in the timing of feather molt among feather tracts. We collected back, breast, and head feathers (approximately 12–15 individual feathers from each feather tract) from each captured bird and stored them in Whirl-paks (Nasco, Modesto, CA, USA) until laboratory analysis. Back feathers collected were restricted to the distinct black triangular patch with white streaks on the upper back. We collected whole blood from each marsh wren from the jugular vein using a heparinized 26 gauge needle and syringe. Blood volume collected was restricted to ≤1% body mass (<100 μL). Blood samples were immediately transferred to 1-mL polypropylene cryovials and stored on wet ice in the field. Samples were transferred to the laboratory within 10 h of collection and stored at –20 °C until analysis.

We searched for marsh wren nests near each mist-netting site and from other areas within the same wetland unit. One egg was randomly chosen from each active nest and collected for mercury analysis. Collected eggs were placed intact into labeled Whirl-paks (Nasco), which were placed in egg cartons for protection, and stored on wet ice in the field. UTM coordinates were recorded at the nest site for each collected egg using a hand-held GPS unit. Eggs were transferred to a laboratory refrigerator within 10 h of collection. Within five days of collection, we measured total egg weight to the nearest 0.01 g on an electronic balance and measured egg length and width to the nearest 0.01 mm using digital calipers. Eggs were then opened, and the contents were emptied into a sterile 30-mL polypropylene jar and frozen at –20 °C until mercury determination. Total content weight was measured to the nearest 0.01 g on an electronic balance. Total egg mass, content mass, length, and width measurements were used to derive fresh wet weight mercury concentrations of eggs (see below).

**Mercury Determination.** As described in Ackerman et al.<sup>22,23</sup> and Ackerman and Eagles-Smith,<sup>24</sup> we processed and analyzed all whole blood, feather, and egg samples for total mercury (THg) at the U.S. Geological Survey, Davis Field Station Environmental Mercury Lab on a Milestone DMA-80 Direct THg Analyzer (Milestone, Monroe, CT, USA) following U.S. Environmental Protection Agency Method 7473.<sup>25</sup> THg concentrations are a reliable proxy for methylmercury (MeHg) concentrations in bird eggs, with an average of 96% of Hg in the MeHg form.<sup>26</sup> THg concentrations were recorded on a wet weight (ww) basis for blood and on a fresh weight

(fw) basis for feathers. Egg contents were dried and then completely homogenized using a mill and mortar and pestle. THg concentrations in eggs were determined on a dry weight (dw) basis and then converted into a fresh wet weight (fww) egg concentration using egg moisture content, an egg volume coefficient of 0.491 derived from house wren (*Troglodytes aedon*) eggs,<sup>27</sup> and an egg density coefficient of 1.031 reported for passerine eggs.<sup>28</sup> Methods and equations for determining fresh wet weight egg mercury concentrations are provided in Ackerman et al.<sup>26</sup> Quality assurance measures included analysis of two certified reference materials per batch (either fish protein (DORM-3) or dogfish liver (DOLT-3) by the National Research Council of Canada, Ottawa, Canada). Recoveries ( $\pm$ SE) for certified reference materials were  $102.2 \pm 1.3\%$  ( $n = 8$ ) for analyses of blood batches,  $104.4 \pm 1.0\%$  ( $n = 13$ ) for feather batches, and  $97.1 \pm 1.4\%$  ( $n = 7$ ) for egg batches. Absolute relative percent difference for all duplicates averaged  $9.3 \pm 2.7\%$  for feathers and  $2.3 \pm 0.6\%$  for eggs.

**Statistical Analyses.** We used general linear models (PROC GLM, SAS/STAT software, release 9.2, SAS Institute, Cary, NC, USA) to analyze variation in THg concentrations in marsh wren tissues. We used a natural log transformation of THg concentrations for all tissues to improve normality of residuals and to meet the assumption of general linear models.

There were three phases to our analysis. First, we examined whether THg concentrations were correlated between body tissues for six pairwise comparisons: back feathers vs blood, head feathers vs blood, breast feathers vs blood, head feathers vs back feathers, breast feathers vs back feathers, and breast feathers vs head feathers. For each comparison, we evaluated a suite of four *a priori* models potentially describing THg concentrations in the dependent tissue type. These models consisted of all combinations of the variables age–sex class (adult male, adult female, or juveniles), THg concentration of the predictor tissue type, and the interaction between age–sex class and THg concentration of the predictor tissue type. For each candidate model set, we also evaluated a null model that included the intercept and variance.

Second, we examined whether age–sex class and wetland unit influenced THg concentrations in marsh wren blood, feathers, and eggs. For blood and feathers, we again evaluated a suite of four *a priori* models for THg concentrations of each dependent tissue type (blood, back feathers, breast feathers, and head feathers). We evaluated all combinations of the predictor variables age–sex class and wetland unit, including an interaction term. For each candidate set, we also evaluated a null model that included the intercept and variance. For eggs, we evaluated two *a priori* models: a model in which egg THg concentrations varied by wetland unit and a null model that included the intercept and variance.

Lastly, we evaluated the correlation between blood or feather (separate analyses for back, head, and breast feathers) THg concentrations and egg THg concentrations. Only blood and feather samples from adults were considered in these analyses (i.e., juvenile birds were excluded). Moreover, it was necessary to link blood and feather samples with corresponding egg samples based on proximity of nests to bird capture locations. We did this at two spatial scales: at the wetland unit scale (which was defined using pre-existing levee boundaries for each wetland unit), and at a smaller scale typical of a marsh wren's home range (which was based solely on proximity of nests to bird capture locations and published accounts of marsh wren territory size). For the wetland unit scale, we calculated the

mean blood and mean feather THg concentrations of marsh wrens by wetland unit and paired these values with the mean THg concentration of all marsh wren eggs (1 per nest) collected within the corresponding wetland unit. For the home range scale analysis, we calculated the mean blood and mean feather THg concentrations for all marsh wrens at a given capture site. We then paired these values with the mean THg concentration of all marsh wren eggs (1 per nest) collected within 200 m of the capture site (see SI for details).

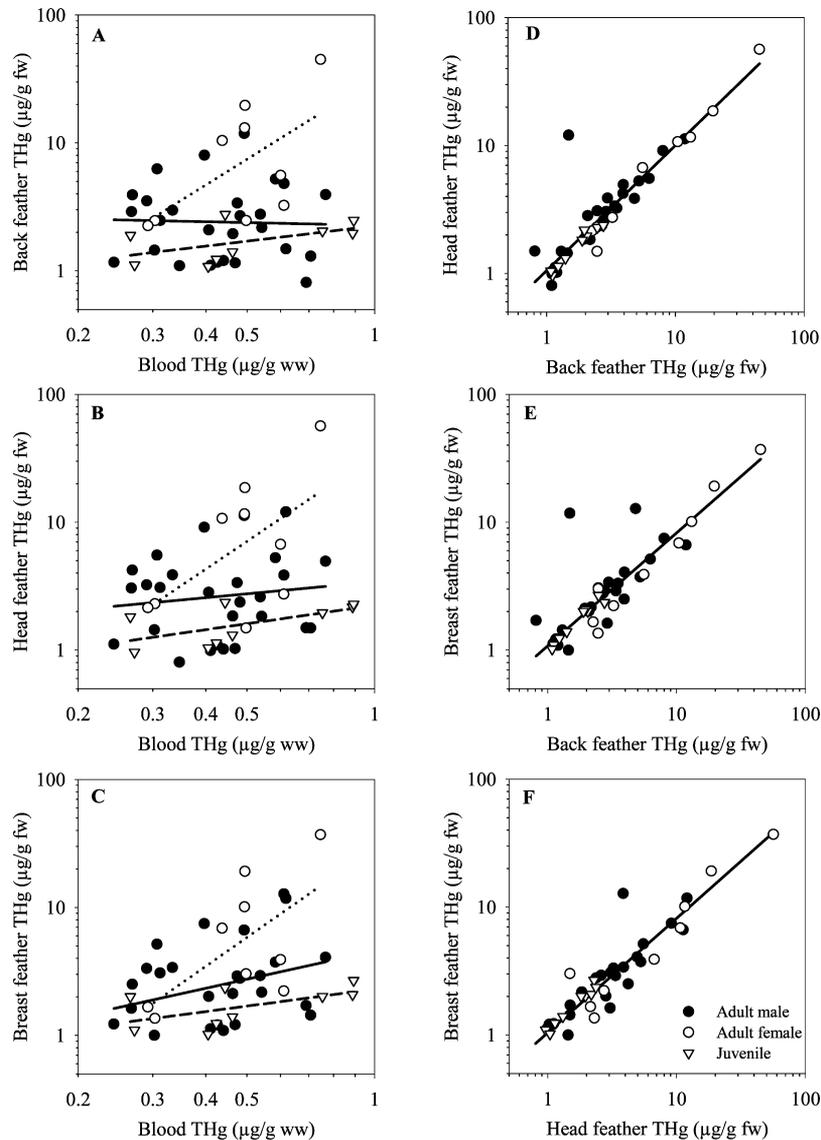
At each spatial scale, we used a general linear model to evaluate the relationship between the dependent variable (mean blood or mean feather THg concentration) and the predictor variable (mean egg THg concentration). Sex composition of sampled birds varied among capture sites and wetland units. To account for the potential effect of varying sex composition among sites on mean blood or mean feather THg concentration, we also evaluated a model in which mean blood or mean feather THg concentration was a function of mean egg THg concentration and the proportion of adult males in the tissue sample. For each candidate set, we included a null model that contained only the intercept and variance.

For all three phases of our analysis, we evaluated the relative support of models using an information–theoretic approach<sup>29</sup> and Akaike's Information Criterion corrected for small sample size ( $AIC_c$ ; see SI for details). We report all THg concentrations in the text as the geometric mean and the 95% confidence intervals (CI) based on the back-transformed least-squares mean and 95% CI from the model output.

## RESULTS

We captured and collected blood and feathers from 45 marsh wrens ( $n = 27$  adult males,  $n = 9$  adult females,  $n = 9$  juveniles) and we collected 40 marsh wren eggs at the BRMBR. Overall geometric mean THg concentrations and 95% CI were  $0.45 \mu\text{g/g}$  ww for blood (0.27–0.77),  $2.76 \mu\text{g/g}$  fw for back feathers (1.10–13.12),  $2.65 \mu\text{g/g}$  fw for breast feathers (1.02–12.75),  $2.84 \mu\text{g/g}$  fw for head feathers (0.99–12.04), and  $0.09 \mu\text{g/g}$  fww for eggs (0.05–0.19).

**Mercury Correlations among Marsh Wren Blood and Feathers.** In evaluating correlations with blood, THg concentrations of back feathers were best explained by a model including blood THg concentrations, age–sex class, and their interaction (see SI Table 1A). This model accounted for 39% of the Akaike model weights. A model with only the variable age–sex class and the additive model blood THg concentrations + age–sex class also were competitive ( $\Delta AIC_c \leq 0.9$ ), accounting for 36% and 25% of the Akaike model weights, respectively. THg concentrations of head and breast feathers were best explained by the additive model blood THg concentrations + age–sex class, accounting for 46% and 59% of the Akaike model weights, respectively (see SI Tables 1B–1C). A model including blood THg concentrations, age–sex class, and their interaction also was competitive ( $\Delta AIC_c \leq 1.3$ ), accounting for 29% and 30% of the Akaike model weights, respectively. For back and head feathers, the variable age–sex class was considerably more important than blood THg concentrations in describing feather THg concentrations (back feathers:  $\sum w_{\text{age–sex class}} = 1.0$ ,  $\sum w_{\text{blood THg}} = 0.64$ ; head feathers:  $\sum w_{\text{age–sex class}} = 0.99$ ,  $\sum w_{\text{blood THg}} = 0.76$ ), whereas for breast feathers, blood THg concentrations were nearly as important as age–sex class (breast feathers:  $\sum w_{\text{age–sex class}} = 0.96$ ,  $\sum w_{\text{blood THg}} = 0.93$ ). For back feathers, the top model was only 1.09 times more likely than the age–sex class only model,

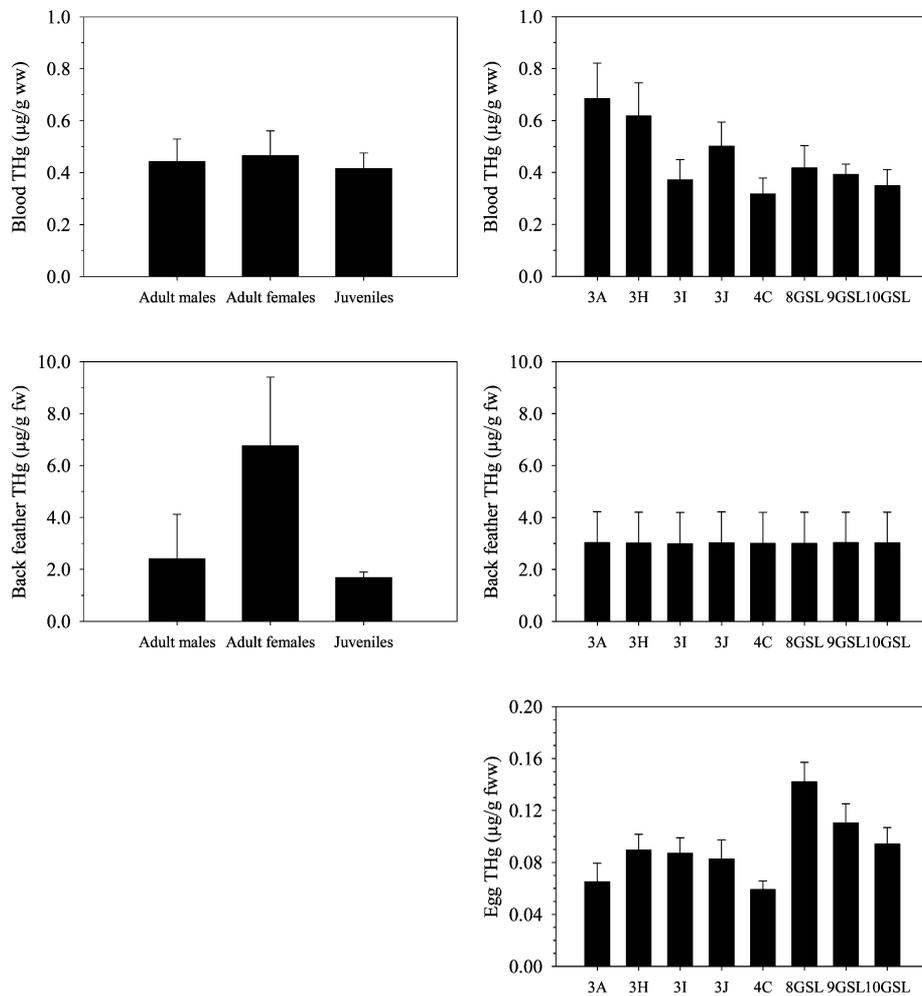


**Figure 1.** Although poor overall, adult females exhibited stronger correlations between (A) back feather, (B) head feather, and (C) breast feather total mercury (THg) concentrations ( $\mu\text{g/g}$  fresh weight, fw) and blood THg concentrations ( $\mu\text{g/g}$  wet weight, ww) than did adult male or juvenile marsh wrens at the Bear River Migratory Bird Refuge, Great Salt Lake, Utah in 2011. (D) Head feather and back feather, (E) breast feather and back feather, and (F) breast feather and head feather THg concentrations ( $\mu\text{g/g}$  fw) were highly correlated among all age–sex classes. Solid circles denote adult males, open circles denote adult females, and white triangles denote juveniles. In A–C, solid lines, stippled lines, and dashed lines represent regressions of adult males, adult females, and juveniles, respectively. In D–F, solid lines represent the regression for all birds combined.

and for head feathers the top model was only 1.93 times more likely than the age–sex class model. Conversely, for breast feathers, the top model was 9.1 times more likely than the age–sex class only model.

These results indicate little relationship between blood THg concentrations and back or head feather THg concentrations, but that blood THg concentrations appeared to be more strongly correlated with breast feather THg concentrations. Although correlations between THg concentrations in blood and feathers were weak overall, THg concentrations among all three feather types were more strongly correlated to blood THg concentrations for adult females and juveniles than for adult males (back feather vs blood: adult males  $R^2 = 0.01$ , adult females  $R^2 = 0.36$ , juveniles  $R^2 = 0.28$ ; head feather vs blood: adult males  $R^2 = 0.02$ , adult females  $R^2 = 0.33$ , juveniles  $R^2 = 0.38$ ; breast feather vs blood: adult males  $R^2 = 0.09$ , adult females  $R^2 = 0.40$ , juveniles  $R^2 = 0.33$ ; Figure 1A–1C).

Unlike correlations between THg concentrations in feathers and blood, THg concentrations were highly correlated among the three feather types (head feather vs back feather:  $R^2 = 0.84$ ; breast feather vs back feather:  $R^2 = 0.74$ ; breast feather vs head feather:  $R^2 = 0.86$ ; Figure 1D–1F). For comparisons among the three feather types, the most parsimonious model was always the other feather type's THg concentration only (see SI Tables 1D–1F). No other models provided a good fit to the data (all  $\Delta\text{AIC}_c \geq 2.9$ ), and the top model with just the other feather type's THg concentration accounted for 79%, 84%, and 88% of the model weights for correlations between head feathers and back feathers, breast feathers and back feathers, and breast feathers and head feathers, respectively. Age–sex class was not important in explaining correlations in THg concentrations among feather types (head feather vs back feather:  $\sum w_{\text{age-sex class}} = 0.21$ ; breast feather vs back feather:  $\sum w_{\text{age-sex class}} = 0.16$ ; breast feather vs head feather:  $\sum w_{\text{age-sex class}} = 0.12$ ).

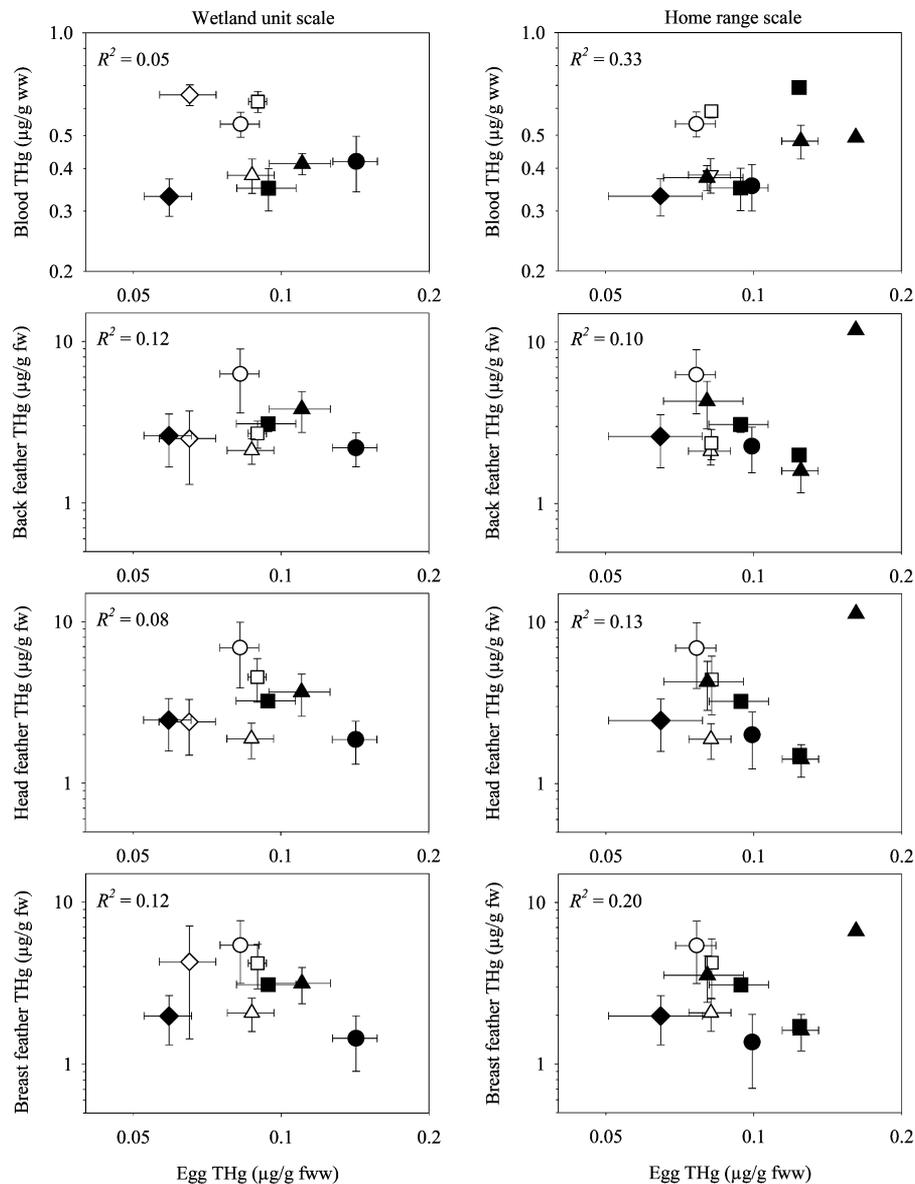


**Figure 2.** Model-averaged least-squares means + standard error (SE) total mercury (THg) concentrations in marsh wren blood and back feathers by age–sex class (left column) and wetland unit (right column) at Bear River Migratory Bird Refuge, Great Salt Lake, Utah in 2011. Also shown are the least-squares means + SE THg concentrations in marsh wren eggs by wetland unit. Sampling different tissues yields differing interpretations of the wetland units and age–sex classes of birds that are most at risk to mercury contamination.

**Variation in Marsh Wren Blood, Feather, and Egg Mercury by Wetland Unit and Age–Sex Class.** Variation in marsh wren blood THg concentrations was best explained by a model that included only the wetland unit variable ( $w_i = 0.63$ ; see SI Table 2A). An additive model containing the variables wetland unit and age–sex class also was competitive ( $\Delta AIC_c = 1.1$ ). However, wetland unit was a much more important predictor of blood THg concentrations ( $\sum w_{\text{wetland}} = 1.00$ ) compared to age–sex class ( $\sum w_{\text{age-sex class}} = 0.37$ ). Model-averaged results indicated that marsh wren blood THg concentrations were greater in a cluster of wetland units at the north end of the BRMBR (units 3A, 3H, 3I, and 3J), than in other wetland units along the margin of the Great Salt Lake (units 4C, 8GSL, 9GSL, and 10GSL; Figure 2). Conversely, model averaged results indicated no difference in blood THg concentrations among age–sex classes (geometric mean and 95% CI: adult males  $0.44 \mu\text{g/g ww}$ ,  $0.32\text{--}0.61$ ; adult females  $0.47 \mu\text{g/g ww}$ ,  $0.33\text{--}0.65$ ; juveniles  $0.42 \mu\text{g/g ww}$ ,  $0.32\text{--}0.53$ ; Figure 2). Among wetland units where both adult males and adult females were captured ( $n = 5$ ), average male and female blood THg concentrations were correlated ( $R^2 = 0.76$ ), indicating that blood mercury concentrations among wetland units varied similarly between sexes.

Unlike THg concentrations in blood, THg concentrations in back, head, and breast feathers were best explained by a model that included only the variable age–sex class (see SI Tables 2B–2D). This model accounted for 98%, 97%, and 90% of the model weights and was 76.8, 64.6, and 9.8 times more likely than the next best models for back feathers, head feathers, and breast feathers, respectively. Adult females had the highest feather THg concentrations (geometric mean and 95% CI: back feathers  $6.77 \mu\text{g/g fw}$ ,  $4.20\text{--}10.92$ ; head feathers  $6.39 \mu\text{g/g fw}$ ,  $3.70\text{--}11.02$ ; breast feathers  $5.22 \mu\text{g/g fw}$ ,  $3.02\text{--}9.03$ ), followed by adult males (back feathers  $2.41 \mu\text{g/g fw}$ ,  $1.89\text{--}3.28$ ; head feathers  $2.63 \mu\text{g/g fw}$ ,  $1.92\text{--}3.61$ ; breast feathers  $2.46 \mu\text{g/g fw}$ ,  $1.79\text{--}3.37$ ), and juveniles (back feathers  $1.68 \mu\text{g/g fw}$ ,  $1.04\text{--}2.72$ ; head feathers  $1.59 \mu\text{g/g fw}$ ,  $0.92\text{--}2.74$ ; breast feathers  $1.67 \mu\text{g/g fw}$ ,  $0.97\text{--}2.89$ ). Unlike for blood, there was no evidence of an effect of wetland unit on feather THg concentrations (Figure 2). For each feather type, including the wetland unit variable greatly reduced model fit, and models with wetland unit accounted for only 1% of the model weights.

Variation in THg concentrations in eggs was best explained by a model that included the wetland unit variable (see SI Table 2E), similar to the result for variation in blood THg concentrations. However, opposite the result for THg concentrations in blood, THg concentrations in eggs were



**Figure 3.** Mean total mercury (THg) concentrations in marsh wren blood ( $\mu\text{g/g}$  wet weight, ww) or feathers ( $\mu\text{g/g}$  fresh weight, fw) were poorly correlated with mean THg concentrations in marsh wren eggs ( $\mu\text{g/g}$  fresh wet weight, fww) at Bear River Migratory Bird Refuge, Great Salt Lake, Utah in 2011. Although still weak, correlations between THg concentrations in marsh wren blood and THg concentrations in marsh wren eggs were relatively stronger when examined at the smaller home range scale of analysis (right-hand column) than the larger wetland scale of analysis (left-hand column). For both scales, the different symbols match individual wetland units. Error bars denote one standard error.

slightly greater in wetland units along Great Salt Lake (units 8GSL, 9GSL, and 10GSL) and lower in other wetland units of the BRMBR (Figure 2).

**Using Mercury Concentrations in Blood and Feathers to Predict Mercury Concentrations in Eggs. Wetland Unit Scale.** We collected 40 marsh wren eggs from eight wetland units where we also captured adult marsh wrens. In evaluating the correlation between mean blood or mean feather THg concentrations and mean egg THg concentrations at the wetland unit scale, the null model (intercept + variance) performed best (see SI Tables 3A–3D). This model accounted for 88%, 89%, 88%, and 82% of the model weights for blood, back feather, head feather, and breast feather THg concentrations comparisons to egg THg concentrations, respectively. Models in which mean blood, back feather, head feather, or breast feather THg concentrations were a function of mean egg

THg concentrations received little support (all  $\Delta\text{AIC}_c \geq 3.2$ ). Models in which the proportion of adult males (PropAdult-M) was included also performed poorly (all  $\Delta\text{AIC}_c \geq 10.1$ ), indicating no effect of this variable on the relationship between mean blood or feather THg concentrations and mean egg THg concentrations. Least-squares means derived from the global additive model (mean egg THg + PropAdult-M) revealed flat to even slightly negative relationships between mean blood or feather THg concentrations and mean egg THg concentrations at the wetland unit scale, and all correlations were very poor (all  $R^2 \leq 0.12$ ; Figure 3).

**Home Range Scale.** Of the 40 eggs collected, 23 eggs were within 200 m of one of 10 marsh wren capture sites within seven wetland units. In addition to excluding samples from juvenile birds from the analysis, we excluded samples from three adult birds captured at two sites where no corresponding

eggs were collected within 200 m. Thus, in comparing THg concentrations of marsh wren blood or feathers with egg THg concentrations at the home range scale of analysis, we had a sample of 33 birds and 23 eggs collected at 10 sites within seven wetland units. Overall geometric mean THg concentration and 95% CI of these 23 eggs was  $0.09 \mu\text{g/g fww}$  ( $0.06\text{--}0.16$ ), similar to the overall geometric mean of  $0.09 \mu\text{g/g fww}$  ( $0.05\text{--}0.19$ ) of all 40 eggs.

As with the analysis at the scale of the wetland unit, mean blood THg concentration at the scale of the marsh wren's home range was best explained by the intercept only model (see SI Table 3E). However, a model that included mean egg THg concentration as a predictor variable was competitive ( $\Delta\text{AIC}_c = 1.5$ ), suggesting a stronger relationship between mean blood THg concentrations and mean egg THg concentrations at this smaller spatial scale. Mean egg THg concentrations were not related to mean back feather, head feather, or breast feather THg concentrations at the home range scale (all  $\Delta\text{AIC}_c \geq 2.9$ , see SI Tables 3F–3H), similar to our analysis at the larger wetland unit scale. Also similar to the analysis at the scale of the wetland unit, the proportion of adult males in the sample had no influence on the relationship between mean blood or feather THg concentrations and mean egg THg concentrations (all  $\Delta\text{AIC}_c \geq 4.2$ ). Least squares means derived from the global additive model (mean egg THg + PropAdult-M) revealed a slightly positive relationship between mean blood THg concentrations and mean egg THg concentrations (Figure 3), illustrating the relatively stronger correlation between these two variables at the smaller home range scale of analysis (Figure 3). In contrast, correlations between mean feather THg concentrations and mean egg THg concentrations exhibited only slight improvement at the home range scale of analysis and were still poor overall (Figure 3).

## DISCUSSION

Marsh wren blood THg concentrations were poorly correlated ( $R^2 = 0.33$ ) with THg concentrations of eggs collected near the site of adult capture ( $<200$  m), and were not at all correlated ( $R^2 = 0.05$ ) with THg concentrations of eggs collected from within the same wetland unit as where adults were captured. Similarly, marsh wren feather THg concentrations were not correlated with egg THg concentrations at either spatial scale (all  $R^2 \leq 0.20$ ). Mercury biomonitoring programs often assume that blood or feather samples from birds captured within a given area accurately reflect the mercury exposure risk to wildlife reproduction in that area. Indeed, previous studies of obligate piscivores have shown that adult female blood mercury concentrations can be strongly correlated with mercury concentrations of eggs collected from the same territory.<sup>5</sup> Wetlands units often are the scale at which management actions occur, and, accordingly, are the scale at which mercury biomonitoring programs typically operate. In breeding areas, wetlands where bird blood mercury concentrations are found to be higher often are concluded to carry greater risk to bird reproduction. However, contradictory results among different tissue types can complicate interpretation of mercury exposure risk. At the BRMBR, marsh wren blood THg concentrations were greatest in the complex of wetland units nearest to the Bear River inflow (units 3A, 3H, 3I, and 3J). Yet marsh wren eggs exhibited an almost opposite pattern, where THg concentrations in eggs tended to be higher in wetland units along the margin of Great Salt Lake (units 8GSL, 9GSL, and 10GSL; Figure 2). These contradictory results illustrate how

estimates of risk of mercury exposure across a landscape can vary depending on the bird tissue examined. Because decision-making practices can be greatly influenced by how mercury exposure to birds is interpreted,<sup>14</sup> such contradictions may complicate management actions. The fact that adult marsh wren blood THg concentrations were not correlated to egg THg concentrations within wetland units highlights the importance of fully developing species and tissues used as bioindicators of mercury exposure. In particular, when proxy measures, such as blood or feather mercury concentrations of individuals in a given area, are used to gauge potential reproductive harm to birds associated with mercury exposure, understanding how mercury concentrations in these tissues translate to mercury concentrations in eggs should be examined.

Correlations between THg concentrations in marsh wren blood and eggs were poor overall, but the relative strength of the correlations varied by the scale of the comparison. At the wetland unit scale, the correlation between THg concentrations in blood and eggs was extremely poor ( $R^2 = 0.05$ ). At the home range scale, where only egg and blood samples within 200 m of one another were compared, the correlation between THg concentrations in blood and eggs improved but was still weak ( $R^2 = 0.33$ ). Correlations between marsh wren feathers and egg THg concentrations at both the wetland unit and home range scale also were poor (all  $R^2 \leq 0.20$ ; Figure 3). Whereas blood and feather mercury concentrations were poor predictors of egg mercury concentrations at both scales, the fact that the correlations improved, particularly for blood, at the smaller home range scale suggests that taking into account species' spatial ecology may improve the effectiveness of using songbird blood mercury concentrations to index egg mercury concentrations over a given area.

Previous studies have found strong positive correlations between a female's blood mercury concentration and the mercury concentration of eggs in her clutch.<sup>16,17</sup> We specifically did not conduct a maternal transfer study. Rather, our study tested whether the relationship between bird blood (or feather) mercury concentrations and egg mercury concentrations often observed in maternal transfer studies persists when examined in a random sample of birds and eggs within a specific wetland unit or at a smaller home range scale. Regardless of whether mercury concentrations are highly correlated between parents and offspring, the present study shows that sampling bird blood or feathers in a specific area, as is the case for many biomonitoring programs, may not accurately index risk to reproduction in that area in the same way as directly sampling eggs. There are a number of potential reasons why mercury concentrations in bird blood sampled from individuals captured in a specific area may not reflect mercury concentrations of eggs in that area. These include (1) changing diet, with corresponding changing mercury exposure, between the time of egg formation and when birds are captured, (2) sampling of transient individuals exhibiting blood mercury concentrations from other areas, and (3) mercury depuration into eggs temporarily lowering blood mercury concentrations in adult females. Regardless of the ecological reasons, the poor correlation in mercury concentrations between blood and eggs sampled in the same area reveals the potential for error of mercury biomonitoring programs attempting to estimate site-specific risk of mercury to reproduction by sampling blood and feather tissues.

In addition to being poor predictors of egg mercury concentrations, feather mercury concentrations were poor predictors of blood mercury concentrations as well. Although

avian feathers are used as bioindicators of mercury contamination, they typically are poorly correlated with internal tissue mercury concentrations in adult<sup>30</sup> and juvenile birds.<sup>31</sup> However, in species that are nonmigratory and have small annual home ranges, feather mercury concentrations can be highly correlated with blood mercury concentrations. For example, breast feather and head feather mercury concentrations were correlated with blood mercury concentrations in endangered California clapper rails (*Rallus longirostris obsoletus*), a year-round resident species that occupies a small annual home range.<sup>32</sup> Mercury concentrations in body feathers and blood also exhibited a positive correlation in nonmigratory Carolina wrens (*Thryothorus ludovicianus*).<sup>10</sup> We hypothesized that THg concentrations in feathers might be more correlated with THg concentrations in blood in western marsh wrens compared to other species because marsh wrens have small home ranges, occur at Great Salt Lake year-round, and are thought to undergo a single molt annually (a prebasic molt that occurs after the breeding season).<sup>21</sup> Yet, correlations between THg concentrations in marsh wren feathers and blood were consistently poor among adult males ( $R^2 \leq 0.09$ ), and were only slightly better among adult females ( $R^2 \leq 0.41$ ) and juveniles ( $R^2 \leq 0.38$ ). Significant spatial variation in THg concentrations in marsh wren blood, but not feathers, among wetland units at the BRMBR (Figure 2) further illustrates the poor applicability of marsh wren feathers as monitoring tools for assessing local mercury exposure. In addition, THg concentrations in feathers differed significantly among marsh wren age–sex classes, whereas THg concentrations in blood did not. Adult females had the highest THg concentrations in feathers, followed by adult males and juveniles. Our results reaffirm that fully grown feathers have limited usefulness for assessing site-specific mercury exposure and yield very different interpretations of mercury exposure from other tissues, such as blood or eggs.

In conclusion, although frequently used as indices of local mercury exposure, our data demonstrate that songbird blood and especially feather mercury concentrations may not always reflect site-specific mercury risk to reproduction in the same way as egg mercury concentrations. Mercury concentrations in feathers did not reveal differences in exposure risk among sites which was apparent using egg tissue, and feather mercury concentrations were not correlated with blood or egg mercury concentrations. Most importantly, blood and egg mercury concentrations did not consistently identify the same wetland units as having elevated exposure risk, and they were poorly correlated among sampling areas. These results illustrate the potential for contrasting management actions that may be implemented based solely on the specific avian tissue that is sampled, and highlights the importance of developing avian tissues as biomonitoring tools that are specific for assessing local risk of mercury exposure to bird reproduction. Although the nonlethal nature of blood and feather sampling is an attractive alternative to lethal egg collections, assumptions of how different tissue mercury concentrations may be correlated among sites need to be validated before data can be fully interpreted.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Additional details regarding the study site, study species, and model selection procedures; three tables summarizing model

selection results for general linear models. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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