MERCURY EXPOSURE AFFECTS THE REPRODUCTIVE SUCCESS OF A FREE-LIVING TERRESTRIAL SONGBIRD, THE CAROLINA WREN (THRYOTHORUS LUDOVICIanus)

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Abstract.—Despite mounting evidence of mercury accumulation in terrestrial ecosystems, few data exist on how environmental mercury exposure affects reproductive success in free-living songbirds. From 2007 through 2010, we monitored reproductive success of Carolina Wrens (Thryothorus ludovicianus) breeding along the forest floodplain of two mercury-contaminated rivers in Virginia. Using an information-theoretic approach, we found a 34% reduction in nesting success of Carolina Wrens on mercury-contaminated sites when compared with reference sites. Blood mercury concentration of the attending female was a strong predictor of nest success. Birds nesting on contaminated sites were 3x more likely to abandon their nests than birds on uncontaminated reference sites. We report a range of effects concentrations associated with various levels of reproductive impairment; for example, a 10% reduction in nest success corresponded with 0.7 µg g⁻¹ mercury in the blood, 2.4 µg g⁻¹ mercury in body feathers, 3.0 µg g⁻¹ mercury in tail feathers, and 0.11 µg g⁻¹ mercury in eggs. This is the first field study to document the effect of specific adult songbird blood mercury concentrations on breeding performance; our results show that free-living songbirds can suffer negative reproductive effects at relatively low mercury concentrations. Received 13 May 2011, accepted 23 August 2011.

Key words: Carolina Wren, ecotoxicology, mercury, nest success, point-source pollution, Thryothorus ludovicianus, Virginia.

La Exposición al Mercurio Afecta el Éxito Reproductivo de Thryothorus ludovicianus, un Ave Canora Terrestre Silvestre

Resumen.—A pesar de una cantidad creciente de evidencia sobre la acumulación de mercurio en los ecosistemas terrestres, existen pocos datos sobre cómo la exposición ambiental al mercurio afecta el éxito reproductivo de las aves canoras silvestres. Desde 2007 hasta 2010, monitorizamos el éxito reproductivo de individuos de la especie Thryothorus ludovicianus que estaban criando a lo largo de la planicie boscosa de inundación de dos ríos contaminados con mercurio en Virginia. Usando un enfoque basado en teoría de la información, encontramos una reducción del 34% en el éxito reproductivo de T. ludovicianus en sitios contaminados con mercurio, comparado con sitios de referencia. La concentración de mercurio en la sangre de las hembras anidantes predijo adecuadamente el éxito de anidación. Las aves que estaban anidando en los sitios contaminados tuvieron tres veces más probabilidades de abandonar sus nidos que las aves de sitios de referencia no contaminados. Brindamos información sobre una serie de concentraciones con efectos asociados con varios niveles de dificultades reproductivas; por ejemplo, una reducción del 10% del éxito de anidación correspondió a concentraciones de 0.7 µg g⁻¹ de mercurio en la sangre, 2.4 µg g⁻¹ de mercurio en las plumas del cuerpo, 3.0 µg g⁻¹ de mercurio en las plumas de la cola y 0.11 µg g⁻¹ de mercurio en los huevos. Este es el primer estudio de campo que documenta el efecto de concentraciones específicas de mercurio en la sangre de aves canoras adultas sobre su desempeño reproductivo. Nuestros resultados demuestran que las aves canoras silvestres pueden sufrir efectos reproductivos negativos ante concentraciones relativamente bajas de mercurio.

Mercury is a persistent and dispersive environmental contaminant found in many ecosystems around the world. Mercury released from industry often finds its way into aquatic systems, where it has long residence times and can bioaccumulate in aquatic food webs (Evers et al. 2005). Most avian bioaccumulation studies have examined fish-eating species that are directly linked with aquatic ecosystems and eat at high trophic levels (Scheuhammer et al. 2007, Seewagen 2010). The Common Loon

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(Gavia immer) is frequently used as an indicator species because of its status as an upper-level predator with a wholly aquatic diet. Common Loons display ~40% reduction in nest success at blood mercury concentrations of 3.0 μg g⁻¹ (wet weight) (Burgess and Meyer 2008, Evers et al. 2008). It has recently become apparent that mercury can contaminate terrestrial ecosystems, where it biomagnifies up the food chain just as in aquatic ecosystems. Terrestrial songbirds that feed primarily on predatory invertebrates such as spiders can bioaccumulate mercury in their tissues at concentrations similar to piscivorous birds because of biomagnification by invertebrates (Cristol et al. 2008).

Little is known about the effects of mercury exposure on terrestrial-feeding songbirds. Studies looking at the reproductive effects of environmental mercury have focused on Tree Swallows (Tachycineta bicolor), which are a model organism in ecotoxicology (Jones 2003). Tree Swallows living at mercury-contaminated sites exhibited moderate reduction in reproductive success associated with mercury exposure, more so when stressed by drought or heat (Brasso and Cristol 2008, Hallinger and Cristol 2011). However, Tree Swallows are not representative of most songbirds; as migrants they may winter in areas with low environmental mercury, allowing them to depurate mercury taken in during the breeding season, and as aerial insectivores they do not feed on spiders or other terrestrial predatory insects. On the basis of these observations, it is not unexpected that some songbird species bioaccumulate mercury at higher concentrations than Tree Swallows and may be affected more. For example, near a mercury-contaminated river in Virginia, Carolina Wrens (Thryothorus ludovicianus; hereafter “wrens”) had blood and feather mercury concentrations similar to those in aquatic-feeding birds (Cristol et al. 2008) and had elevated mercury levels 140 km downstream from the source of aquatic mercury (Jackson et al. 2011).

Although ecotoxicological studies focused on Tree Swallows are generally designed to compare nest success in treatment groups to controls (Custer et al. 2007, Brasso and Cristol 2008), the analysis of nest success in songbird species other than Tree Swallows brings several added complications. First, for species that do not nest in artificial cavities (e.g., nest boxes), nests are typically discovered at different stages of the nesting cycle, making the duration of time that each nest is under observation—a source of error originally identified by Mayfield (1961, 1975)—likely to influence estimates of success rates. Second, because of logistical constraints, nests in wild populations are often monitored at irregular intervals, necessitating the estimation of dates of fledging or failure. Finally, success of nests in natural sites tends to be influenced by more factors than those in nest boxes. Daily nest survival rates may vary by date or age of nest (Dinsmore et al. 2002, Etterson et al. 2007b) as well as a long list of other factors (Johnson 2007). Few ecotoxicological studies have taken into account the multiple factors, in addition to the toxin under study, that influence nesting success in wild birds.

Current nest-survival estimation methods used by researchers to examine the influences of hypothesized covariates on nest survival implicitly treat all causes of failure as equivalent in their effects (Dinsmore et al. 2002, Shaffer 2004). However, given the diversity of causes of nest failure (Heisey and Patterson 2006) it is logical to expect that covariates that influence rates of failure due to one cause (e.g., predation) may be different from covariates influencing rates of failure due to another cause (e.g., abandonment or adverse weather; Etterson et al. 2007a, b). Although ornithologists have recognized this issue for some time (reviewed by Johnson 2007), generalized software for modeling these competing risks and their relative effects in the presence of discovery bias and irregular monitoring schedules has been lacking.

We modeled nest success using a novel software program that can take into account covariates thought to affect nest survival in wild populations (e.g., mercury exposure, age of nest, time in season, year, and type of nest cavity) and document the relative influence of these factors on various causes of nest failure. Using all of these covariates set within an information-theoretic approach, our goals were to (1) determine the relative influence of mercury exposure compared to other covariates and model the difference in nest survival between contaminated and reference treatment groups; (2) use female blood mercury concentrations to model nest survival based on individual mercury body burdens; (3) compare cause-specific nest failure rates between mercury contaminated and reference populations; and (4) calculate preliminary estimates of mercury concentrations in blood, feathers, and eggs associated with various levels of reproductive failure.

**Methods**

**Study species.**—Wrens are monogamous and territorial passerines that nest up to three times per year in our study region (Haggerty and Morton 1995). Both males and females remain on their territories year round, making them prime candidates to be indicators of contaminants on a small geographic scale. Blood mercury concentrations reflect recent dietary exposure to mercury (French et al. 2010). Feather samples represent exposure to mercury >8 months previous because wrens molt from July through September, after their breeding season is complete; therefore, with the exception of accidentally lost feathers that are regrown within the year, feather values reported here likely reflect exposure during the previous year (Haggerty and Morton 1995, Pyle 1997). Most wrens likely experience similar mercury concentrations year round.

**Study area.**—We monitored wrens along two mercury-contaminated rivers in Virginia: the North Fork Holston and South rivers (Fig. 1). The two rivers are in different watersheds and, historically, both were contaminated with mercury as the result of industrial discharge. The floodplain forest surrounding these rivers is subject to seasonal flooding and is classified as a forested wetland (Cowardin et al. 1979). Given that all monitored territories abutted a river, the birds occupying these territories likely were exposed to dietary uptake of mercury within the floodplain forest.

The North Fork Holston River (NFHR) flows through Smyth and Washington Counties in southwestern Virginia. The NFHR joins the South Fork Holston River to form the Holston River near Virginia’s southern border with Tennessee. Contamination occurred between 1950 and 1972 at a chlor-alkali facility in Saltville, Virginia. We monitored wrens on two sites downstream of the mercury contamination and three reference sites upstream (Fig. 1B). All nests included in our analysis were within 50 m of the shore of the river.

The South River (SR) flows through Rockingham and Augusta counties in central Virginia. The SR joins the North River to form the South Fork Shenandoah River, which ultimately becomes the Shenandoah River. Contamination occurred between
1929 and 1950 at a textile factory in Waynesboro, Virginia. We monitored wren territories at 11 sites downstream of the source of contamination and 11 reference sites, either upstream of the point source along the SR itself (three sites), on a tributary of the SR (Back Creek, six sites), or on the Middle River (two sites) (Fig. 1C). Birds on these reference sites have been shown in other studies to have low levels of mercury exposure consistent with background atmospheric deposition (Brasso and Cristol 2008, Cristol et al. 2008). All nests included in our analysis were within 200 m of the shore of the river.

Field methods.—We erected nest boxes (10 × 10 × 25 cm) or nest tubes (46 × 10 cm diameter) within floodplain forest containing resident wrens on each river to encourage wrens to nest where we could access them. We mounted nest boxes on poles ~1.5 m off the ground and fit them with stovepipe predator guards, but these proved ineffective in our study. We fashioned nest tubes from flexible plastic irrigation pipe with a hole on one end for the bird to enter and a removable cover on top to allow nest checks. We attached the nest tubes directly to trees, ~1 m off the ground, and did not install predator guards.

We monitored nest boxes and tubes starting on 1 April 2007, 10 April 2008, 7 April 2009, and 15 March 2010; we checked the nest boxes and tubes weekly until a nest was initiated and then more often to monitor reproductive success. We did not actively search for nests in natural nest cavities from 2007 through 2009, but if we discovered nests in natural cavities during nest-box checks they were also monitored. In 2010, field teams conducted intensive behavioral observations to find and monitor natural nest cavities. Observers spent 2 to 3 hours week−1 on each territory where no nest had been initiated in a nest box to observe adult behavior and locate natural nest cavities. We checked nests approximately every 1 to 3 days to record the state of the nest, including determining whether the female was incubating, how many eggs or chicks were present, and the approximate age of the nestlings based on size and feathering. In 2010, we also used motion-sensing infrared video cameras (Bushnell Corporation, Overland Park, Kansas) to determine causes of nest abandonment or predation. These motion activated cameras were set up after incubation had started and recorded video in 10-s intervals when activated by movement.

Territorial birds were captured early in the season (before a nest was found) using playback recordings and identified as male by the presence of a cloacal protuberance. Because we wanted blood and feather samples associated with particular nest attempts we recaptured and sampled both parents once a nest was found. In order to minimize the risk of nest abandonment we captured parents at the nest by mist net only after nestlings had hatched (average 7 days posthatch; range: day 1 to day 14). We collected either the two outermost tail feathers or 5 to 10 body feathers sampled from a similar area on the back. We used 26- to 28-gauge needles to puncture the cutaneous ulnar vein of the wing and collected blood in heparinized capillary tubes sealed at both ends with Critocaps and placed in 10-cm³ plastic tubes for protection. We placed samples on ice in a cooler and they were frozen at −25°C within 6 h of collection. We banded the adult birds with a federal metal band and a combination of one or two color bands. Males had already been captured and sexed earlier in the season and we determined females by the presence of a brood patch. In 2009 and 2010, we collected any inviable eggs left after all nestlings hatched or a nest failed, placed them in glass jars, and stored them on ice until taken to the laboratory. The egg contents (albumen and yolk) were extracted from the shell, weighed, and frozen at −25°C within 6 h of collection. We weighed egg contents before and after freezing and the loss of weight was not statistically significant.

Mercury.—Prior to analysis we sprayed each feather with de-ionized water for 1 min to remove surface particles and dried them at low humidity for ≥48 h, thereby returning the cleaned feather to approximately wet weight. We cut up and homogenized the entire
feather (including rachis) prior to analysis. We analyzed all blood and feather samples directly from the thawed collection containers without freeze drying; we report both blood and feather samples as wet weight. We freeze dried egg contents (albumen and yolk) prior to analysis. Because eggs lost little moisture during the freezing process we calculated mercury concentrations using the postfreezing wet weight, as this could be done on the same laboratory balance, reducing instrument-derived variance. Egg mercury concentrations are reported as wet weight. All concentrations are for total mercury, which is a proxy for methylmercury because 95–99% of total mercury found in avian blood, feathers, and eggs consists of methylmercury (Rimmer et al. 2005, Bond and Diamond 2009).

Determination of total mercury concentration of avian tissues occurred at four laboratories: Trace Element Research Lab at Texas A&M University, College Station, using a Direct Mercury Analyzer (2007 blood samples; DMA-80; Milestone, Shelton, Connecticut); Center for Environmental Sciences and Engineering at the University of Connecticut (2008 blood samples, using EPA method 245.6 with a flow injection mercury system; Perkin Elmer, Milford, Connecticut; 2009 and 2010 egg samples, Milestone DMA-80; College of William and Mary, Williamsburg, Virginia (2009 blood samples, Milestone DMA-80); and Biodiversity Research Institute, Gorham, Maine (2010 blood and feather samples, Milestone DMA-80). Avian tissue samples were analyzed over a 4-year period at these four laboratories and quality assurance data for the narrow periods when wren samples were run met acceptable standards (Appendix). In general, before and after every set of 20 samples, two samples each of two standard reference materials (SRMs, DORM, and DOLT; National Research Council, Ontario, Canada), two methods blanks, and two sample blanks were run, and recovery of SRMs was ~100% (Appendix). During the periods of mercury determination we spiked samples of blood or egg expected to have low mercury concentrations with SRM to measure recovery in the appropriate matrix. We recovered close to 100% of the added mercury (Appendix). We included approximately one pair of samples from the same bird with every 20 samples and obtained relative percent differences between duplicates that were consistently ≤10% (Appendix). All blood and feather samples were well above minimum instrument detection limits.

Statistical analysis.—We used EXCEL (Microsoft, Redmond, Washington) and JMP (SAS Institute, Cary, North Carolina) for general statistical analysis. We log transformed blood mercury values to normalize the data and check for normality within the contaminated and reference groups using a Shapiro-Wilk test of normality. We compared blood mercury concentrations for each treatment (contaminated vs. reference) and year combination for all adult (after-hatch-year, AHY) wrens using a one-way analysis of variance (ANOVA) followed by Tukey’s HSD post hoc test. Using inviable eggs collected on the SR in 2009 and 2010, we compared the relationship between attending female blood mercury concentration and average inviable egg mercury concentrations using linear regression.

In 2010, we intensified sampling effort and were therefore able to investigate the relationship between male and female blood mercury concentrations using a linear regression for adult pairs that were caught while attending the same nest. We used the regression calculated from this analysis to create post hoc estimates of female blood mercury for nests at which we had captured the male but were unable to capture the attending female. For all captured adults we explored the relationship between blood and feather mercury, plotting a linear regression between log-transformed blood mercury concentration and log-transformed tail or body feather mercury. All figures show back-transformed data.

As discussed previously, many ecotoxicological studies fail to account for biological covariates known to influence nest success. Several good computer programs (White and Burnham 1999, Dinsmore et al. 2002) and code for statistical software (Stanley 2000, 2004; Shaffer 2004) already exist for estimating daily survival rates as a function of covariates, along with accounting for discovery bias and irregular check schedules. These programs depend on a binomial distribution of outcomes (success or failure) and fewer methods exist for nests with multinomial outcomes (success, depredated, adverse weather, abandonment, etc.), especially when the latter require analysis of covariates. When nests are visited daily, multinomial logistic regression may be used (Thompson and Burnhans 2004). However, when nest visitation is variable the mathematical accounting requires special handling. We therefore used a novel program called MCESTIMATE to estimate daily and overall probabilities of nest failure due to specific causes (competing risks) using the Markov chain algorithms described by Etterson et al. (2007a, b) within a user-friendly graphical interface. It is programmed in MATLAB (Mathworks 2009) and compiled as a stand-alone program. Like other current nest-survival estimation methods, MCESTIMATE is a generalization of Mayfield (1961, 1975) methods for estimating daily probabilities of failure. When nest outcomes are classified binomially, the likelihood function employed by MCESTIMATE is equivalent to that of Johnson (1979) and Bart and Robson (1982), which underlies the nest-survival algorithm in Program MARK (White and Burnham 1999, Dinsmore et al. 2002), and to logistic exposure (Shaffer 2004).

We used MCESTIMATE within an information-theoretic framework to analyze the nest survival data from 2007 through 2010, thus determining whether mercury contamination had more effect than other variables likely to affect nest survival. In addition to mercury contamination we identified five variables that might influence nest survival: year, date in season, time since egg laying, cavity type (box, tube, or natural), and river system (SR or NFHR). We were unable to include individual sites within each river system as a covariate because of low sample size of nests at many sites and the fact that site boundaries were arbitrary, related to access and ownership rather than biology (e.g., nests on the periphery of some sites were closer to nests on other sites than they were to nests on their own sites). To determine the relative effect of each variable we first ran six univariate models and selected the minimal set of covariates that accounted for ≥95% of the model weights. We then used this subset of covariates to create a logical model set to use within an information-theoretic framework (Akaike’s information criterion corrected for small sample sizes; AICc). We evaluated each model within this AICc framework. If multiple models appeared to have similar AICc weights, indicating that more than one model could explain variation in nest success, we model averaged across all models to calculate predicted nest survival rates between treatment and control nests. This
also required specifying values for the other two covariates that occurred in this model set: cavity type (set equal to natural nest) and date (set equal to 24 May, the average clutch initiation date in our sample). We then calculated model-averaged predictions for treatment and control nests using Akaike weights following Burnham and Anderson (2002). We calculated percent reduction from the success of reference nests using the following equation: percent reduction = [(success ref – success cont)/ success ref]. To better understand the effect of cavity type we held treatment group (set equal to reference) and date (set equal to 24 May) constant and model-averaged the effect of cavity type across all models. Effective sample size was calculated following Rotella et al. (2004).

We assumed that all these nests were independent data points because even for known renesting or second clutch attempts nest-box locations had changed. Other nests may have been renests as well if pairs escaped detection during their first attempt or moved into the study area, so we made no attempt to distinguish between nesting attempts and assumed that inclusion of date in season as a covariate would suffice to capture any effect of renesting or double clutching.

In 2010, we intensified effort to obtain blood mercury concentrations for each attending female. The larger sample size allowed us to examine the relationship between individual mercury concentration and reproductive success in 2010 as opposed to comparing reference and contaminated groups. In cases where we were unable to capture attending females we estimated female blood concentrations using the linear regression between territorial pairs described above. We looked at four variables separately—cavity type (box, tube, or natural), date in season, time since laying, and female blood mercury concentration—and selected the minimal set of covariates that accounted for ≥95% of the model weights.

In 2010, we used observations from nest cameras to classify nest fate into several categories: fledged, failed due to predation, and failed due to abandonment. Fledged nests were those observed active late in the nesting cycle (usually days 12–14 posthatch) and then found empty with no sign of disturbance and with parents observed feeding young fledglings. Nests that were depredated had nestlings or eggs missing, and often the nest was disturbed. In some cases we observed the predator on video. Abandoned nests were those in which eggs or nestlings were found left in the nest, with no evidence of a predator. Abandonment may have occurred for multiple, but unknown, reasons (e.g., egg infertility, abnormal incubation or feeding behavior, adult mortality, food availability, weather, or mercury toxicity in eggs or nestlings). We used MCESTIMATE to estimate and compare cause-specific failure rates for these fates between contaminated and reference for 2010 data only.

Effects concentrations.—We used the MCESTIMATE model that calculated nest success at various female blood mercury concentrations as the basis for our extrapolation of effects concentrations in blood, feather, and eggs. Using the three regression equations calculated for the relationship between wren blood mercury concentrations and body feathers, tail feathers, or egg concentrations, we calculated the concentration in each tissue associated with different levels of reproductive failure. We report all error estimates as SE.

Results

Nest sampling.—We monitored 6 wren nests in 2007 along the NFHR and 9 nests in 2008. On the SR, we monitored 29 nests in 2009 and 45 in 2010. All but one nest in 2007 through 2009 were in nest boxes or tubes; in 2010, we monitored 27 natural nests. Clutch initiation dates ranged from 23 March to 7 August. We monitored renesting attempts for the same banded female 19 times (4 in 2008, 2 in 2009, 13 in 2010). Successful nests did not vary in the number of fledglings produced between treatment groups (Kruskal-Wallis, \( \chi^2 = 0.54, \text{df} = 1, P = 0.46 \)) or among years (Kruskal-Wallis, \( \chi^2 = 1.75, \text{df} = 3, P = 0.63 \)). On average, successful nests produced 4.0 ± 0.15 fledglings (range: 1–6, \( n = 49 \)).

Mercury concentrations.—Log transformed blood mercury values for all adult birds were normally distributed within contaminated and reference populations (Shapiro-Wilk Test, contaminated: \( W = 0.990, P = 0.73 \); reference: \( W = 0.988, P = 0.58 \); \( n = 95 \)). There were significant differences between treatment groups and year (one-way ANOVA, \( F = 149.97, \text{df} = 7 \) and 174, \( P < 0.001 \), \( n = 87 \)). Blood mercury concentrations were significantly elevated above reference in each year (Fig. 2). NFHR and SR birds had similar annual patterns of blood mercury concentrations, with the exception of a significant difference between blood mercury concentrations in 2007 (\( \overline{X} = 2.69 \pm 1.1 \mu g g^{-1}, n = 30; \) all territories were <50 m from river) and 2010 (\( \overline{X} = 1.74 \pm 1.1 \mu g g^{-1}, n = 33; \) some territories were 50–200 m from river; Fig. 2). We sampled both sexes in each year, but low sample sizes prevented statistical comparison by sex (Table 1). In 2010 only, we sampled a high proportion of the male wrens associated with nests and found a strong relationship between male and female blood mercury at the same nest (\( F = 385.2, P < 0.001, n = 16; \) Fig. 3).

![Fig. 2. Mean back-transformed adult Carolina Wren blood mercury levels for each treatment group and year (wv = wet weight). The North Fork Holston River was sampled in 2007 and 2008 and the South River was sampled in 2009 and 2010. Different letters indicate significant differences between the groups with analysis of variance (P < 0.05) and numbers indicate sample size. Error bars represent back-transformed 95% confidence intervals.](image-url)
Using the three top covariates (treatment group, cavity type, and date), we designed a set of eight candidate models that we hypothesized could explain the variation in survival and found support for many models—including the null model—within the AICc framework (Table 2). Although four models—including the null model—had ΔAICc scores <2, the total weight of models that contained treatment group was 0.88, more than twice the total weight of models that included either date (0.38) or cavity type (0.24). After running each model with these parameters set to average values we model-averaged the effect of treatment across all models (Burnham and Anderson 2002). The overall 30-day nest survival for reference nests was 0.602 ± 0.111 (n = 42), whereas that for nests at contaminated sites was 0.398 ± 0.129 (n = 46), a reduction of 34%.

Because cavity type was the covariate with the second highest weight, we also wanted to test whether there were differences in survival between the three cavity types. We observed both predation and abandonment in all types of nest cavities. We saw little difference in overall nest success among the different cavity types, with natural nests (0.602 ± 0.110, n = 28) having slightly higher survival than tube nests (0.534 ± 0.122, n = 14) or box nests (0.552 ± 0.101, n = 46).

We used MCESTIMATE to model nest survival in 2010 on the basis of individual female blood mercury concentrations because that was the only year for which we had adequate sample size (effective sample size = 581 days). The female blood mercury concentrations used in the model ranged from 0.07 to 3.48 µg g⁻¹ (X = 1.11 ± 0.17, n = 40). The maximum female blood mercury concentration for a captured female in 2010 was 3.22 µg g⁻¹ (n = 30; Table 1); for nests where we could not catch the attending female (n = 10) we estimated female blood mercury concentrations from that of the male. Four nests were excluded because of missing blood mercury data (two contaminated and two reference). We again ran univariate models to determine the relative influence of covariates (female mercury, cavity type, date, and time since laying). The top two covariates accounted for >95% of the model weights (female mercury w₁ = 0.93; cavity type w₂ = 0.03) and so were selected to be included in the model set while the lower-ranked covariates (time since laying w₃ = 0.03; date w₄ = 0.01) were excluded. From female mercury and cavity type covariates we created four candidate models and found support for the two top-ranked models (Table 3). Models containing female blood mercury concentration

![Graph](image)

**Fig. 3.** A plot of the relationship between blood mercury concentrations of adult Carolina Wren pairs at the same nest in 2010 in Virginia (ww = wet weight). Adult Carolina Wren male and female (sampled from the same nest) blood mercury concentrations show a strong positive linear relationship (r² = 0.97, F = 385.2, P < 0.001).

In 2010, we sampled tail and body feathers (presumably grown at the end of the previous breeding season) from adult wrens and found a strong relationship between blood and body feather mercury (r² = 0.88, F = 133.1, P < 0.001, n = 21; Fig. 4) and a significant but weaker relationship between mercury in blood and tail feathers (r² = 0.56, F = 64.5, P < 0.0001, n = 53). Although we had a small sample size, we found a strong relationship between attending female blood mercury concentration and average inviable egg total mercury (r² = 0.89, F = 41.51, P = 0.001, n = 7).

**Nest survival.—**Our analysis of nest survival in MCESTIMATE was based on an effective sample size of 1,371 days. Three covariates accounted for >95% of the model weights: treatment group (w₁ = 0.81), cavity type (w₂ = 0.11), and date (w₃ = 0.04). Time since laying (w₄ = 0.02), river system (w₅ = 0.02), and year (w₆ < 0.001) contributed little additional explanatory power and were excluded from the subsequent analyses (data not shown).

**Table 1.** Mean blood mercury concentrations for female and male adult Carolina Wrens in each year and treatment group in Virginia.

<table>
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<tr>
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<td>10</td>
<td>0.38</td>
<td>0.20</td>
<td>0.84</td>
<td>0.08</td>
<td>4</td>
<td>0.34</td>
<td>0.25</td>
<td>0.70</td>
<td>0.11</td>
</tr>
<tr>
<td>SR 2010</td>
<td>C</td>
<td>11</td>
<td>2.13</td>
<td>0.67</td>
<td>3.22</td>
<td>0.96</td>
<td>22</td>
<td>1.74</td>
<td>0.68</td>
<td>3.65</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>21</td>
<td>0.21</td>
<td>0.11</td>
<td>0.55</td>
<td>0.07</td>
<td>24</td>
<td>0.19</td>
<td>0.08</td>
<td>0.42</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*aNFHR = North Fork Holston River, SR = South River.

*bTreatment group: C = contaminated, R = reference.*
accounted for 93% of weights and models containing cavity type accounted for 24% of weights.

Within the top-ranked model (effect of female blood mercury alone) we found an effect of female blood mercury on daily nest survival (on the multinomial logit scale, $\beta = 0.564 \pm 0.072$). We transformed this beta estimate into predicted nest success (assuming a 30-day nesting cycle) based on hypothesized female mercury body burden and found that as female blood mercury increased, modeled nest survival decreased (Fig. 5A). Because we were interested in how individual blood mercury concentrations affected nest survival in relation to individuals with no mercury exposure, we calculated percent reduction in nest success compared with modeled females with 0 µg g$^{-1}$ blood mercury concentrations and found that nest success decreased as blood mercury concentration increased (Fig. 5B).

Competing risks.—In 2010, out of 19 monitored contaminated nests, 9 fledged, 3 were depredated, and 7 were abandoned.

On reference sites, 21 nests fledged young, 2 were depredated, and 2 others were abandoned. Video revealed predation by American Black Bears (*Ursus americanus*) and activity around failed nests by other potential nest predators, including Northern Raccoons (*Procyon lotor*), Virginia Opossums (*Didelphis virginiana*), and Coyotes (*Canis latrans*). We suspect some instances of snake predation but did not capture this on video. Of the 9 abandoned nests, 4 occurred during the laying–incubation stage and 5 occurred during the nestling stage. In some cases the parents renested later in the season, which indicates that adult mortality had not caused abandonment, but we do not have any other information to explain abandonment.

By evaluating competing risks in MCESTIMATE we found that cause-specific nest failure differed between contaminated and reference sites (effective sample size = 615 days). The top-ranked model—which had $\Delta AIC = 3$ x the support of the second-ranked model—revealed an important effect of treatment on the rate of abandonment but no important effect of treatment on predation (Table 4). Within the top-ranked model contaminated nests were nearly 4 x more likely to be abandoned (abandonment$_{cont} = 0.507 \pm 0.111$, abandonment$_{ref} = 0.131 \pm 0.081$) but there was little

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**Table 2.** $AIC_c$ ranking for treatment-group candidate model set for Carolina Wren nests monitored between 2007 and 2010 on the North Fork Holston and South rivers in Virginia. Models are ranked by increasing $\Delta AIC_c$ scores, showing that the treatment effect appears in the top three ranked models and accounts for >80% of model weights.

<table>
<thead>
<tr>
<th>Model</th>
<th>$K^a$</th>
<th>$\Delta AIC_c^b$</th>
<th>$w_c^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>2</td>
<td>0.00</td>
<td>0.39</td>
</tr>
<tr>
<td>Treatment + date</td>
<td>3</td>
<td>0.20</td>
<td>0.32</td>
</tr>
<tr>
<td>Treatment + cavity</td>
<td>4</td>
<td>1.00</td>
<td>0.14</td>
</tr>
<tr>
<td>Cavity</td>
<td>3</td>
<td>1.99</td>
<td>0.05</td>
</tr>
<tr>
<td>Null</td>
<td>1</td>
<td>2.34</td>
<td>0.04</td>
</tr>
<tr>
<td>Cavity + treatment + date</td>
<td>5</td>
<td>2.42</td>
<td>0.03</td>
</tr>
<tr>
<td>Date</td>
<td>2</td>
<td>3.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Cavity + date</td>
<td>4</td>
<td>3.83</td>
<td>0.01</td>
</tr>
</tbody>
</table>

$^a$Number of parameters.

$^b$Scaled $AIC_c^b$; $\Delta AIC_c^b = 0.00$ is interpreted as the best fit to the data among all models.

$^c$Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.

**Table 3.** $AIC_c$ model rankings for female blood mercury burden analysis for Carolina Wren nests monitored in 2010 on the South River, Virginia. Models are ranked by increasing $\Delta AIC_c$ scores, showing that the effect of female blood mercury appears in the top two ranked models and accounts for >90% of model weights.

<table>
<thead>
<tr>
<th>Model</th>
<th>$K^a$</th>
<th>$\Delta AIC_c^b$</th>
<th>$w_c^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>2</td>
<td>0.00</td>
<td>0.71</td>
</tr>
<tr>
<td>Mercury + cavity</td>
<td>4</td>
<td>1.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Null</td>
<td>1</td>
<td>2.70</td>
<td>0.05</td>
</tr>
<tr>
<td>Cavity</td>
<td>3</td>
<td>3.38</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$^a$Number of parameters.

$^b$Scaled $AIC_c^b$; $\Delta AIC_c^b = 0.00$ is interpreted as the best fit to the data among all models.

$^c$Weight of evidence interpreted as a proportion. Weights across all models sum to 1.00.
The relationship between MCESTIMATE-modeled Carolina Wren nest survival and female blood mercury concentration for nests found in 2010 in Virginia. (A) Predicted Carolina Wren nest success over their 30-day nest cycle in relation to female blood mercury concentration when other covariates were held constant (date = 24 May, nest cavity = natural). Error bars indicate SE. Dotted portion of the line indicates model extrapolation past observed female blood mercury concentrations. (B) Percent reduction in nest success (from nest survival at 0 µg g\(^{-1}\)) in relation to female blood mercury concentration. Blood mercury concentrations associated with 10% increments of reduction in nest success are shown.

**FIG. 5.**

difference in predation between treatment groups (predation\(_{\text{Cont}} = 0.145 \pm 0.061\); predation\(_{\text{Pred}} = 0.196 \pm 0.078\)).

Effects concentration.—We found that the blood concentration associated with 10% reduction in nest success was 0.70 µg g\(^{-1}\), which correlated to 2.4 µg g\(^{-1}\) mercury in body feathers, 3.0 µg g\(^{-1}\) mercury in tail feathers, and 0.11 µg g\(^{-1}\) mercury in egg tissue (Table 5). Extrapolating our model to higher mercury values predicts 99% reduction in reproductive success at blood concentrations of 5.6 µg g\(^{-1}\), body feather concentrations of 12.8 µg g\(^{-1}\), tail feather concentrations of 19.5 µg g\(^{-1}\), and egg concentrations of 0.97 µg g\(^{-1}\) (Table 5).

**TABLE 4.** AIC\(_c\) model ranking for Carolina Wren nest survival in 2010 along the South River, Virginia, when nest survival was allowed to vary by cause between models. Models are ranked by increasing ΔAIC, scores, showing a strong effect of abandonment within treatment groups, but not predation.

<table>
<thead>
<tr>
<th>Model</th>
<th>(K)</th>
<th>ΔAIC(_c)</th>
<th>(w_t)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abandonment (treatment) Predation (.)</td>
<td>3</td>
<td>0.00</td>
<td>0.74</td>
</tr>
<tr>
<td>Abandonment (treatment) Predation (treatment)</td>
<td>4</td>
<td>1.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Abandonment (.) Predation (.)</td>
<td>2</td>
<td>3.23</td>
<td>0.03</td>
</tr>
<tr>
<td>Abandonment (.) Predation (treatment)</td>
<td>3</td>
<td>4.48</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(K\) Number of parameters.
ΔAIC\(_c\) = 0.00 is interpreted as the best fit to the data among all models.

**TABLE 5.** Carolina Wren blood, feather, and egg mercury effects concentrations (ww = wet weight) associated with MCESTIMATE-modeled reduction in nest success. Results based on data collected in 2010 from nests along the South River in Virginia.

<table>
<thead>
<tr>
<th>Reduction in nest success (^a)</th>
<th>Blood mercury (µg g(^{-1}), ww)</th>
<th>Body feather mercury (µg g(^{-1}), ww) (^b)</th>
<th>Tail feather mercury (µg g(^{-1}), ww) (^c)</th>
<th>Egg mercury (µg g(^{-1}), ww) (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>0.7</td>
<td>2.4</td>
<td>3.0</td>
<td>0.11</td>
</tr>
<tr>
<td>20%</td>
<td>1.2</td>
<td>3.4</td>
<td>4.7</td>
<td>0.20</td>
</tr>
<tr>
<td>30%</td>
<td>1.7</td>
<td>4.5</td>
<td>6.4</td>
<td>0.29</td>
</tr>
<tr>
<td>40%</td>
<td>2.1</td>
<td>5.3</td>
<td>7.7</td>
<td>0.36</td>
</tr>
<tr>
<td>50%</td>
<td>2.5</td>
<td>6.2</td>
<td>9.1</td>
<td>0.43</td>
</tr>
<tr>
<td>60%</td>
<td>2.9</td>
<td>7.1</td>
<td>10.4</td>
<td>0.50</td>
</tr>
<tr>
<td>70%</td>
<td>3.3</td>
<td>7.9</td>
<td>11.8</td>
<td>0.57</td>
</tr>
<tr>
<td>80%</td>
<td>3.8 (^e)</td>
<td>9.0</td>
<td>13.5</td>
<td>0.66</td>
</tr>
<tr>
<td>90%</td>
<td>4.4 (^e)</td>
<td>10.3</td>
<td>15.5</td>
<td>0.76</td>
</tr>
<tr>
<td>99%</td>
<td>5.6 (^e)</td>
<td>12.8</td>
<td>19.5</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^a\)Calculated using MCESTIMATE, comparing probability of fledging at least 1 young at 0 µg g\(^{-1}\) to the probability of fledging at least 1 young at each contaminated blood concentration.
\(^b\)Calculated using the regression equation [body feather Hg] = 2.1407974[blood Hg] + 0.8531665.
\(^c\)Calculated using the regression equation [tail feather Hg] = 3.3762108[blood Hg] + 0.6427166.
\(^d\)Calculated using the regression equation [egg Hg] = 0.1748381[blood Hg] + 0.76.

**DISCUSSION**

Carolina Wrens breeding in two mercury-contaminated watersheds in Virginia had 34% lower nest success compared with nearby reference sites without histories of industrial mercury contamination. At an individual level, females with higher blood mercury concentrations had lower nest success; across the range from 0 to 4.0 µg g\(^{-1}\), modeled success decreased by 10% on average for each projected 0.50 µg g\(^{-1}\) increase in blood mercury concentration. Our analyses consistently ranked mercury contamination as a leading predictor of nest success, more so than date in season, year, cavity type, age of nest, or river system. By using new software to model nest success on the basis...
of competing risks we were able to show that nests on contaminated sites were 3× more likely to fail because of abandonment, whereas both treatment groups experienced equal levels of nest predation.

We estimated approximately 60% nest success on reference sites and 40% nest success on contaminated sites, both of which are higher than the only other known published estimate for this species: 26% of nests in northwestern Alabama produced at least one fledgling (n = 118; Haggerty and Morton 1995). In our female blood mercury analysis, we estimate that females without methylmercury in their diet should fledge young ~80% of the time. This is higher than expected for a songbird, but similar rates have been reported in other cavity-nesting species (Martin and Li 1992, Etterson et al. 2007b).

Because we determined the mercury concentrations of female parents (or estimated it from male concentrations) at the time of nesting, we were able to model the response of nest survival to female mercury concentration in 2010. This is the first study, to our knowledge, that documents a correlation between blood mercury concentration and reduction in nest survival in a free-living bird population. Several recent field studies have compared the reproductive success of birds exposed to environmental mercury with that of an unexposed reference group (Custer et al. 2007, Brasso and Cristol 2008). These studies, however, did not consider nest success on the basis of individual blood mercury concentration.

The maximum blood mercury concentration for wrens in 2010 was 3.22 µg g⁻¹ (wet weight) for females and 3.65 µg g⁻¹ (wet weight) for males. Although it is possible to extrapolate the model to show almost complete reproductive failure (99%) at 5.6 µg g⁻¹ in the blood, these extrapolations should be viewed with caution. It is possible that complete reproductive impairment occurs at lower concentrations than those predicted by our model; individuals with blood mercury levels higher than those that we sampled may be unable to establish territories or find mates, rendering their reproductive output effectively zero, which is a finding that cannot be shown in our study.

In other orders of birds, blood mercury concentrations are affected by depuration of mercury into eggs (Becker 1992, Monteiro and Furness 2001, Kennamer et al. 2005, French et al. 2010) or into feathers during molt (Nichols et al. 2010). We believe that the timing of blood sample collection within the nesting cycle is not likely to have caused variability in blood mercury concentrations in wrens at this heavily contaminated South River site. For example, mercury transfer from blood into eggs by Tree Swallows breeding along a mercury-contaminated stretch of the South River did not decrease in the amount of mercury transferred to each subsequent egg laid, which indicates that intake of mercury through prey was high enough to maintain body burden despite the depuration into eggs (Brasso et al. 2010). Methylmercury depuration through molt also complicates interpretation of blood mercury levels, but the wren is a year-round resident and does not start molting until after their nestlings have fledged. Therefore, molt would not have eliminated blood mercury to growing feathers until after our sampling efforts.

Abandonment rates were ~3× higher at contaminated than at reference sites. There are several putative mechanisms by which this abandonment may have occurred (some seen in other mercury studies and some speculative), but we are unable to distinguish between them without further study. Some parents that abandoned nests may have succumbed to lethal effects of mercury, although determining which behavioral or physiological effect is not possible. At the sublethal level, adults may have exhibited abnormal incubation or feeding behavior that led to nest loss. Common Loons exposed to environmental mercury displayed aberrant incubation behavior, leaving eggs unattended more often when mercury concentrations were elevated (Evers et al. 2008). Nestlings may have behaved abnormally, for example vocalizing less before hatching or begging less, eliminating cues necessary to stimulate parental behavior. Finally, mercury can cause outright embryo mortality in songbirds, in which case the documented abandonments would be best explained as a response to egg inviability rather than a cause (Heinz et al. 2009). Other mechanisms are possible, but identifying abandonment rather than predation as a differential cause of reduced nest survival is an important next step in focusing future studies of the effects of mercury on songbird reproduction. Dosing studies may be an important next step, because they can eliminate the risk of predation to focus on the mechanisms of abandonment. In a dosing study of American Kestrels (Falco sparverius), researchers also found decreased nest success but were unable to conclude whether differences were caused by abnormal parental behavior or disrupted egg and chick development (Albers et al. 2007).

Wrens accumulate mercury to higher concentrations than other songbirds breeding in floodplain forests, likely because of their heavy reliance on spiders in their diet (Cristol et al. 2008). Because spiders feed at high trophic levels, they bioaccumulate mercury to higher concentrations than many other invertebrate prey species. Because wrens remain on territories year round, they may be one of the more at-risk species, but the effects of mercury reported here are biologically significant enough that migrants, and species feeding lower on the food chain, may also be affected. Although the sites studied here were both industrial point sources, songbirds living in areas remote from industry can also accumulate mercury concentrations comparable to those that have effects on wren reproduction. These include Rusty Blackbirds (Euphagus carolinus; Edmonds et al. 2010), Nelson’s Sparrows (Ammodramus nelsoni; Winder and Emslie 2011), and Saltmarsh Sparrows (A. caudacutus; Lane et al. 2011).

Conclusions.—Our results have important implications with regard to future regulation of mercury pollution and mitigation of previously contaminated sites. Our finding that terrestrial songbirds exposed to mercury exhibited high rates of nest abandonment, leading to substantial reduction in return on reproductive effort, suggests that aquatic mercury pollution may harm terrestrial songbirds in floodplain forest habitats near many of the thousands of water bodies subject to mercury fish-consumption advisories in the United States (see water.epa.gov/scitech/swguidance/fishshellfish/fishadvisories) and worldwide. This study is the first to link specific mercury concentrations in songbirds to a quantifiable reduction in nest success, at least in part because of increased abandonment of nests. Understanding the ramifications of mercury contamination for other species and regions requires further investigation, particularly for species found in habitats sensitive to methylmercury production or experiencing greater-than-usual physiological demands such as long-distance migration.
We thank the field crews and numerous landowners who allowed us access to their property, along with two anonymous reviewers who offered helpful comments on the manuscript.

**Acknowledgments**

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**Literature Cited**


Associate Editor: J. Jones

Appendix. Quality assurance information (means ± SD; sample size [n] in parentheses) for total mercury analysis at four laboratories: William and Mary (W&M), Center for Environmental Science and Engineering (UCONN), Trace Elements Research Lab (TERL), and Biodiversity Research Institute (BRI).

<table>
<thead>
<tr>
<th></th>
<th>W&amp;M</th>
<th>UCONN</th>
<th>TERL</th>
<th>BRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duplicatesa</td>
<td>8.1 ± 10.7 (24)</td>
<td>7.3 ± 76.0 (5)</td>
<td>3.1 ± 2.5 (12)</td>
<td>5.0 ± 6.9 (9)</td>
</tr>
<tr>
<td>Percent recovery:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DORM-2</td>
<td>101.2 ± 5.0 (47)</td>
<td>NA</td>
<td>98.2 ± 1.9 (12)</td>
<td>NA</td>
</tr>
<tr>
<td>DORM-3</td>
<td>NA</td>
<td>NA</td>
<td>102.6 ± 3.2 (10)</td>
<td>NA</td>
</tr>
<tr>
<td>DOLT-3</td>
<td>100.3 ± 1.6 (22)</td>
<td>93.0 ± 2.8 (2)</td>
<td>102.2 ± 2.3 (12)</td>
<td>NA</td>
</tr>
<tr>
<td>DOLT-4</td>
<td>95.8 ± 2.8 (23)</td>
<td>103.5 ± 3.5 (2)</td>
<td>NA</td>
<td>94.6 ± 1.5 (10)</td>
</tr>
<tr>
<td>Tissue spike</td>
<td>100.0 ± 1.5 (10)</td>
<td>94.6 ± 4.3 (5)</td>
<td>97.7 ± 2.6 (24)</td>
<td>NA</td>
</tr>
</tbody>
</table>

aRelative percent difference between two duplicate blood samples.

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