Suppressed Adrenocortical Responses and Thyroid Hormone Levels in Birds near a Mercury-Contaminated River

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Much of the research on sublethal, adverse effects of mercury (Hg) has focused on impairment of neurological function and reproduction in fish and fish-eating vertebrates. Here we examined the associations between Hg and endocrine function (adrenocortical responses and plasma thyroid hormone concentrations) of insectivorous tree swallow nestlings adjacent to a Hg-contaminated river and nearby reference rivers in Virginia. Nestlings from the contaminated sites had blood Hg concentrations that exceeded those from the reference sites by more than an order of magnitude (354 ± 22 vs 17 ± 1 ppb wet weight). A regression of age and Hg concentrations suggested dietary Hg at the contaminated sites exceeded the nestlings' capacity to eliminate Hg through deposition into growing feathers. Although blood Hg concentrations among nestlings at the contaminated sites were lower than those typically associated with abnormal behavior or altered physiology in young birds, adrenocortical responses, plasma triiodothyronine, and thyroxin concentrations were suppressed, relative to reference levels, by the end of the nestling period. These results suggest that (1) Hg may disrupt endocrine systems of terrestrial avian young and (2) adverse effects of Hg on endocrine systems may be most evident once endocrine axes are fully developed.

Introduction

Mercury (Hg) is one of the most widespread pollutants threatening the health of both humans and wildlife globally (*1*). Until recently, research on Hg pollution has primarily focused on aquatic environments including fish and fish-eating vertebrates (*2*). However, studies now indicate that high levels of Hg can be found even in insectivorous songbirds in forest habitats (*3, 4*). Most research on adverse effects of Hg has focused on impacts to the nervous system and reproduction (*5*). Although direct empirical data are limited, some studies suggest Hg can also disrupt endocrine systems in laboratory mice (*6, 7*), such as the thyroid and adrenocortical hormonal axes, that are important for metabolism, development, and thermoregulation.

Similar to other pollutants, Hg may act as an environmental stressor to organisms. When animals perceive a stressor, they often secrete glucocorticoids (cortisol and corticosterone) from their adrenals. This response redirects energy and behavior from processes accessory to life (e.g., reproduction) to those necessary for survival (*8*). Thus, the relative reactivity of the hypothalamic-pituitary-adrenal (HPA) axis (i.e., adrenocortical reactivity) has been used to assess impacts of environmental stressors such as unpredictable weather, human disturbance, and pollution on animals (*9*). Laboratory studies on fish indicate Hg exposure can alter HPA axis reactivity depending on exposure conditions (*10*−*12*). Although young are generally more sensitive to Hg than adults (*1*), little is known about the effects of Hg on the HPA axis during early developmental stages.

Mercury may also disrupt the hypothalamic-pituitary-thyroid (HPT) axis. Thyroid hormones play critical roles in metabolism and normal development of the nervous system and in thermoregulation (*13, 14*). Two forms of thyroid hormones, triiodothyronine (T3) and thyroxine (T4) are stored and released from the thyroid glands. Triiodothyronine is the more potent form and most T4 is converted to T3 in peripheral tissues by deiodinases. Some evidence suggests Hg can affect secretion and/or deiodination of thyroid hormones in mice and humans (*6, 7, 15*). In addition, dietary intake of Hg delayed tail resorption during amphibian metamorphosis (*16*), a process mediated by thyroid hormones. Although adverse effects of Hg on the HPT axis have not been studied in birds, it is possible that some developmental and behavioral abnormalities seen in avian young after Hg exposure (*17*) are due to disrupted thyroid hormone levels.

To explore the potential relationships between Hg exposure and both adrenal and thyroid function during development, we sampled nestling tree swallows (*Tachycineta bicolor*) along the Hg-contaminated South River near Waynesboro, VA. During the 1930s and 1940s, Hg, in the form of mercuric sulfate, entered the river from an industrial plant in Waynesboro. For a comparison, additional nestlings were sampled upstream from the contamination source and from two nearby rivers where the only known source of Hg is atmospheric deposition. At both sites, we subjected swallow nestlings to a standardized handling stress protocol to assess adrenocortical reactivity (*18*) and plasma T3 and T4 concentrations, using the hormone concentrations to assess the functionality of the two endocrine axes. Because adrenocortical responses and thyroid hormone levels change through early development, we measured these responses during early, middle, and late stages of the nestling period. We hypothesized that (1) adrenocortical responses, T3, and T4 would increase with increasing age, and (2) both adrenal and thyroid functions would be suppressed in the contaminated sites compared to the reference sites.

Materials and Methods

Study species and site. The tree swallow is a migratory, altricial passerine. During the nestling period, adult tree swallows forage mostly within 100−200 m of their nest and feed aerial insects to the young, including Diptera and Hemiptera (*19*). Tree swallow nestlings sampled in our study

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occupied nest boxes in Hg-contaminated and reference sites along the South, Middle, and North Rivers in Virginia (Supporting Information (SI) Figure S1). These nest boxes have been monitored since establishment in 2005 and were located within 200 m of the South River shoreline. Nests along the South River downstream from the point source of Hg contamination were designated as contaminated. Those along the portion of the South River upstream from the contamination source, and on the nearby Middle and North Rivers, were pooled to collectively form our reference nests because nestling blood Hg concentrations were low and equivalent among the sites. A recent analysis of surface water and sediment at the South River and the reference sites confirmed that Hg was the primary contaminant at the South River and that organochlorine pesticides, polycyclic aromatic hydrocarbons, and other trace metals, such as cadmium, copper, chromium, lead, selenium, and zinc, were generally low (*20*). To examine how age might interact with Hg, the ~20-day nestling period was divided into three sample age classes: early (days 3−6, period of eye opening), middle (days 7−12, period of most rapid mass gain *21, 22*), and late (days 13−17, when mass plateaus prior to fledging). Nestling age was estimated through a combination of observing hatching during routine nest monitoring and a comparison of physical characteristics to known-aged nestlings. Within each site, nests were selected for sampling during one of the three age classes based on availability and timing. In order to avoid possible habituation to handling, nestlings were sampled only once. We sampled 10−13 nests of each age class at both contaminated and reference sites ($N = 72$ nests).

Sample Collection. In May−June 2007, nestlings in each nest were divided into three groups; one nestling was used to assess the adrenocortical response, one nestling for blood Hg concentration, and the remaining 2−3 nestlings for analysis of thyroid hormones. Runts (i.e., individuals falling outside of the usual size distribution) were excluded so that each nestling used was representative of the developmental stage and body condition of the majority of nestmates. The small body size of nestlings precluded measurements of Hg and hormone levels on blood from the same individual. Since interindividual variation in blood Hg is generally low within nests (*23*−*25*), the Hg concentration of blood from one nestling was considered to represent Hg exposure of nest mates. To investigate the magnitude of adrenocortical responses in tree swallow nestlings, we used the standardized capture and handling protocol described by Wingfield (*18*). Briefly, baseline blood samples were collected within three minutes of opening the nest box. The nestling was then placed in an opaque cloth bag. After 30 min of total handling time (i.e., from the time the nest box was opened), a second sample was collected to measure stress-induced levels of corticosterone. Blood samples for Hg and thyroid hormone analysis were collected within one hour of disturbing a nest box. Body mass and lengths of tarsus and third primary feather (P3) were measured before nestlings were returned to their nest.

Blood samples for all analyses were collected with heparinized capillary tubes after puncturing the alar vein with a 26-gauge needle. Nestlings were captured between ~6:15am and ~2:00 pm and blood samples were stored on ice for less than ~10 h before processing. Blood samples for corticosterone and thyroid hormones were centrifuged at 12 000 rpm (15 300 g) at room temperature for eight minutes. Plasma was then collected and frozen at −25 °C before being transferred to −80 °C until assayed. Blood samples for Hg analysis were not centrifuged but were frozen in capillary tubes and stored at −25 °C until assayed.

Corticosterone Assay. Plasma corticosterone levels were measured using enzyme immunoassay kits (Cat No. 901-097, Assay Designs). The assay was optimized for tree swallow nestling plasma using a technique described in Wada et al. (*26*). The results from the optimization procedure indicated the optimal conditions to be a 1:20 dilution with 3% steroid displacement buffer for tree swallow nestling plasma.

For the assays, each 96-well plate had a standard curve ranging from 15.63 to 2000 pg/mL with standards assayed in triplicate. In addition, it contained a 500 pg/mL standard in triplicate to obtain interplate variability and bird plasma samples in duplicate. The detection limit of the assay was 9.1 pg/well (1.81 ng/mL). Samples were haphazardly distributed across and within plates. The detection limit of the plate was used when samples fell under the limit, which occurred in 7 out of 144 samples. Intra- and interassay variation was calculated as the average coefficient of variation across all samples within each plate and of 500 pg/mL standards across plates, respectively. Intra- and interassay variation was 7.93 and 6.45%, respectively.

Thyroid Hormone Assay. Plasma T4 and T3 concentrations were measured using radio-immunoassay following the protocol described in McNabb and Hughes (*27*). The assays were first validated using pooled tree swallow nestling plasma from other nestlings in the same sites. All samples were haphazardly distributed and analyzed within a single assay each for T4 and T3. Detection limits of the assays for T4 and T3 were 1.25 and 0.125 ng/mL, respectively. None of the samples fell below the detection limit. However in one sample, no precipitate formed so it was excluded from further analysis. Intra-assay variations calculated as the average coefficient of variation across all samples for T4 and T3 were 7.88 and 11.48%, respectively.

Mercury Analysis. Measurement of total Hg was used as a proxy for the highly bioavailable methylmercury (MeHg), based on the assumption that nearly all mercury in avian blood is MeHg. To validate this assumption we determined the proportion of total Hg that was MeHg in 15 tree swallow blood samples collected from the same study sites. Methylmercury was analyzed by Quicksilver Scientific (Lafayette, CO) using acidic thiourea leaching and mercury-thiourea liquid chromatography coupled to cold vapor atomic fluorescence spectrometry (HgTu/LCCVAFS), which separates monomethyl (CH_3Hg+) from mercuric (HgII) mercury by the charges on their respective thiourea complexes; online cold-vapor generation follows separation with an absolute instrument detection limit of 0.40 pg for CH_3Hg+.

Nestling blood mercury concentrations were analyzed for total mercury using a DMA 80 direct mercury analyzer (Milestone, Inc.) at the College of William and Mary. Every 20 samples included two samples of each standard reference material (DORM-2/DORM-3 and DOLT-3), a method blank, a sample blank, and a sample replicate (i.e., second capillary tube from the same bird). Mean percent recoveries of the standard reference materials were 93.2 ± 1.4% (DORM-3), and 97.1 ± 1.5% (DOLT-3). Percent difference between the duplicate samples was 1.5 ± 1.1% ($n = 10$ samples). Detection limit of the assay was 2.6 ppb. We present blood mercury concentrations as ppb wet weight (ww).

Data Analysis. Statistical analyses were performed using SPSS 15.0 and SAS 9.1. The effect of site type on blood Hg concentrations in nestlings was analyzed using ANCOVA with age as a covariate. Blood Hg concentrations were log-transformed prior to analysis. The effects of site type on body mass and lengths of tarsus and P3 were analyzed using a nonparametric Wilcoxon test, blocked by three age classes. In our statistical analyses of hormone levels, blood Hg could not be treated as a continuous variable (e.g., regression analysis) because blood Hg concentrations were separated by an order of magnitude between the two types of sites, with no overlap (see Figure 1b). Therefore, we treated contaminated or reference sites as a categorical variable in all subsequent analyses of hormones. Because hormone levels fluctuate over the course of a day, we included time of day

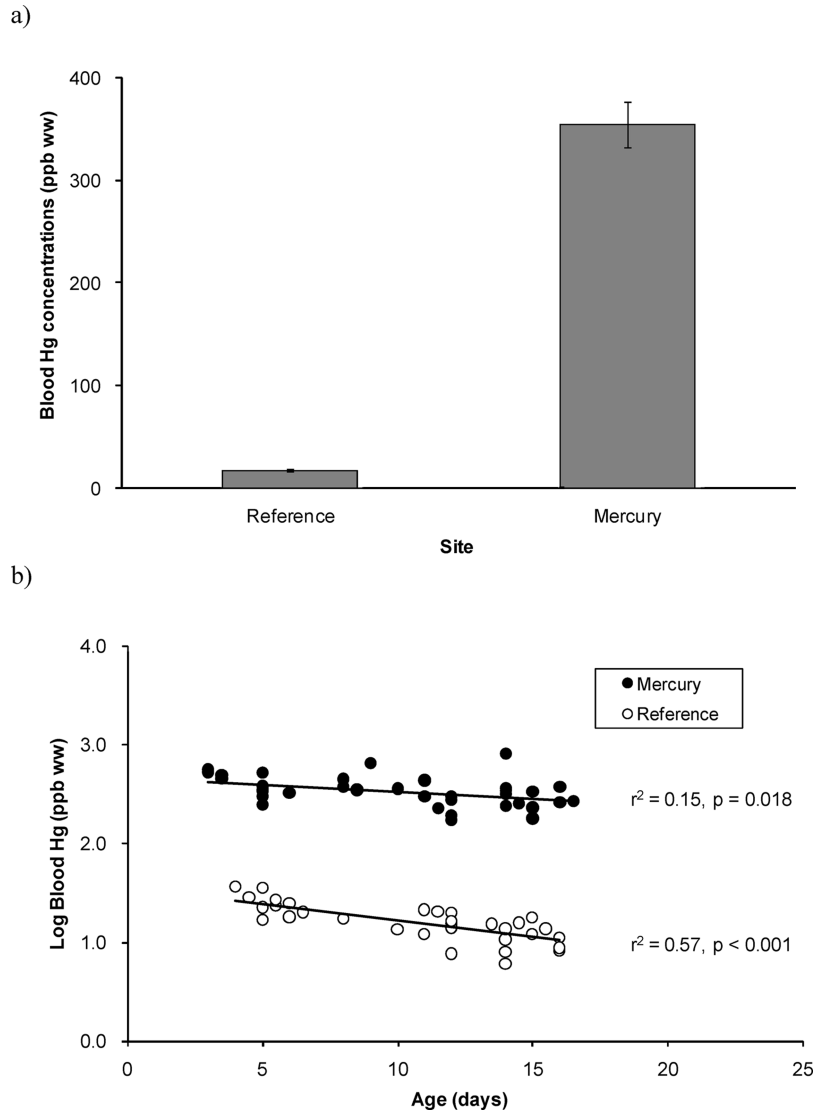

FIGURE 1. Blood Hg concentrations (ppb ww) in tree swallow (*Tachycineta bicolor*) nestlings from reference and Hg-contaminated sites in Virginia. Panel a depicts mean blood Hg concentrations (ppb ww) ±1 SE in reference and contaminated (mercury) sites. Panel b depicts a correlation between nestlings' age and log blood Hg concentrations. Open and filled circles represent reference and contaminated sites, respectively.

as a covariate in the initial ANCOVA models for adrenocortical responses and thyroid hormones. When time of day was not significant ($p > 0.15$) it was removed from the analysis and ANOVA was employed. The effects of age and site type on adrenocortical responses (log-transformed) were determined using two-way repeated-measures ANOVA, followed by Tukey HSD. Due to unequal variances, effects of age and site type on plasma T4 levels were analyzed using a mixed model approach to ANCOVA (SAS PROC MIXED) while two-way ANCOVA was used for T3 levels (log-transformed). Data are presented as mean ±1 SE.

Results

Blood Mercury Concentrations and Body Measures. Average total blood mercury concentrations in swallow nestlings from our reference sites were 17.32 ± 1.20 ppb, which is similar to or lower than those found in other North American bird samples without known Hg point sources (*24, 25, 28*). Because MeHg constituted 95.4 ± 2.6% of total Hg in swallow blood samples from the study sites, total Hg provides a good approximation of MeHg. Tree swallow nestlings from contaminated sites had mean total blood Hg concentrations that were 20 times higher than nestlings from reference sites (site: $F = 174.64$, $p < 0.001$, Figure 1a and b). Blood Hg concentrations decreased with increasing age of nestlings, although the strength of the relationship depended on site type (age: $F = 39.40$, $p < 0.001$, Figure 1b). A significant site-by-age interaction indicated that blood Hg concentrations declined more rapidly with age in reference nestlings than in contaminated nestlings (site × age: $F = 6.81$, $p = 0.011$). Mass, tarsus, and P3 lengths of nestlings in the three age classes did not differ between the two types of sites (mass: $X^2 = 0.82$, $p = 0.365$; tarsus: $X^2 = 2.13$, $p = 0.145$; P3: $X^2 = 1.93$, $p = 0.165$).

Adrenocortical Responses in Tree Swallow Nestlings. Baseline corticosterone concentrations fell within a narrow range, except for late-stage nestlings at the contaminated sites, which had baseline corticosterone concentrations twice as high as all other groups (Figure 2a). Thirty minutes of restraint elicited a significant elevation in plasma corticosterone in all age classes (restraint: $F = 224.71$, $p < 0.001$), and these adrenocortical responses to handling and restraint increased with age (age: $F = 7.29$, $p = 0.001$; restraint*age: $F = 7.14$, $p = 0.002$). Posthoc comparisons indicated that adrenocortical responses elicited by restraint in early stage nestlings were significantly lower than responses in the late-

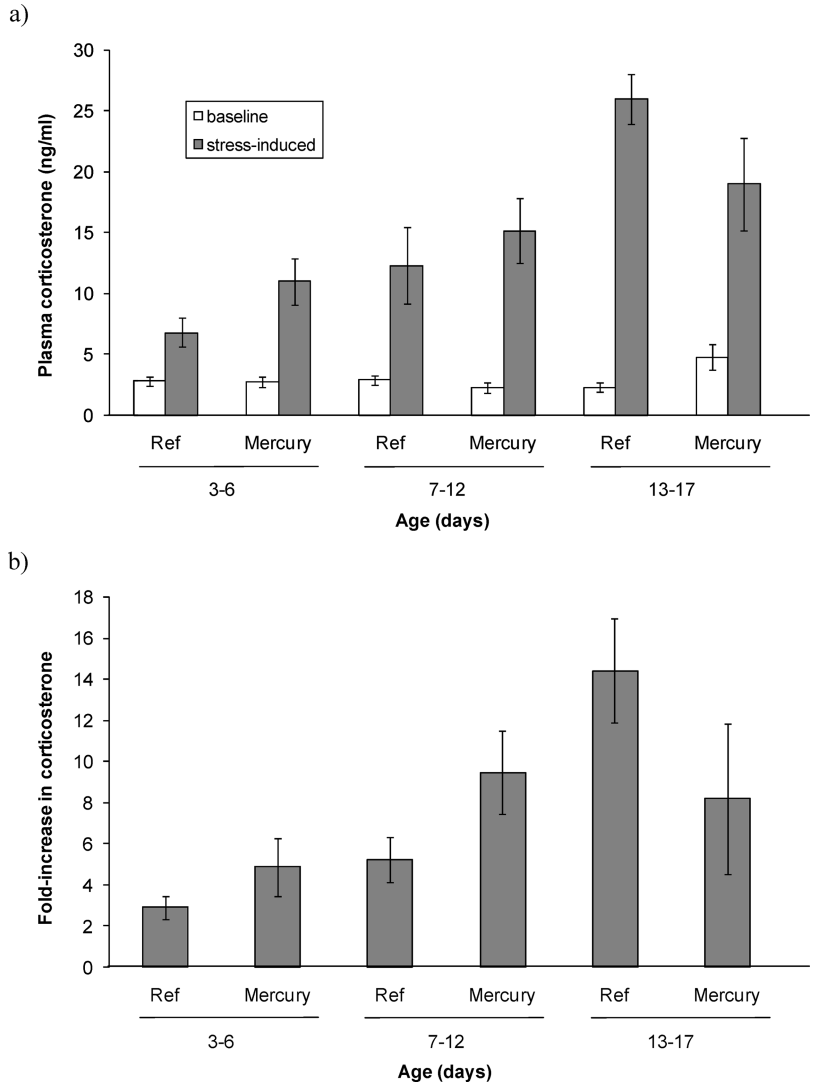

FIGURE 2. Adrenocortical responses were measured from tree swallow (*Tachycineta bicolor*) nestlings in reference (ref) and Hg-contaminated (mercury) sites in Virginia. (a) The baseline sample was collected within 3 min of capture to represent a baseline corticosterone level. Nestlings were then held in opaque cloth bags for 30 min and the stress-induced sample was collected. The figure represents mean plasma corticosterone levels (ng/mL) ±1 SE in days 3−6, 7−12, and 13−17 nestlings. Open and filled bars represent baseline and stress-induced levels of corticosterone, respectively. (b) Fold-increase in corticosterone following handling stress was calculated as stress-induced divided by baseline corticosterone levels. The figure represents mean fold-increase ±1 SE *n* = 12 each.

stage nestlings (Figure 2a). Plasma corticosterone levels also differed based on site type, however, these differences were dependent on nestling age and restraint (restraint × site × age: $F = 5.49$, $p = 0.006$). The difference between reference and contaminated birds was most apparent in late-stage nestlings, as those from the contaminated sites had 103% higher baseline and 27% lower stress-induced levels compared to late-stage nestlings from the reference sites. Fold-increase in corticosterone is shown to illustrate the three-way interaction (Figure 2b).

Thyroxine and Triiodothyronine in Tree Swallow Nestlings. Plasma T4 concentrations differed by site but the difference depended on nestling age and the time of day that sample was taken (site: $F = 0.88$, $p = 0.354$; time of day: $F = 15.41$, $p < 0.001$; site*age: $F = 3.32$, $p = 0.049$; time of day*site*age: $F = 3.87$, $p = 0.033$; Figure 3a). Specifically, plasma T4 levels were similar between contaminated and reference site in days 3−12 nestlings, but were depressed in the contaminated sites in the late age class. In addition, T4 levels were highest in the morning and gradually declined over the course of the sampling day (from 6:27 a.m. to 2:16 p.m.). We also found an ontogenetic increase in T4 after days 3−6 (age: $F = 11.66$, $p <= 0.001$; time of day × age: $F = 5.24$, $p = 0.012$).

Plasma T3 concentrations showed the opposite diurnal pattern to T4, increasing over the course of the sampling day (time of day: $F = 3.96$, $p = 0.051$, power = 0.50). Unlike T4, we did not detect an ontogenetic increase in T3 with age (age: $F = 2.59$, $p = 0.083$, power = 0.50). Mean plasma T3 levels in the three age classes were consistently 15−40% lower in the contaminated sites than the reference sites (site: $F = 8.60$, $p = 0.005$; age × site: $F = 1.04$, $p = 0.36$, power = 0.22, Figure 3b).

Discussion

To our knowledge, this is the first study to suggest disruption of multiple endocrine functions by Hg in wild animals. Tree swallow nestlings from the contaminated sites had blood Hg concentrations that exceeded those from the reference sites by more than an order of magnitude. Compared to the reference sites, adrenocortical responses, plasma T3, and T4 concentrations were altered in the contaminated sites. Site differences in adrenocortical responses were complex.

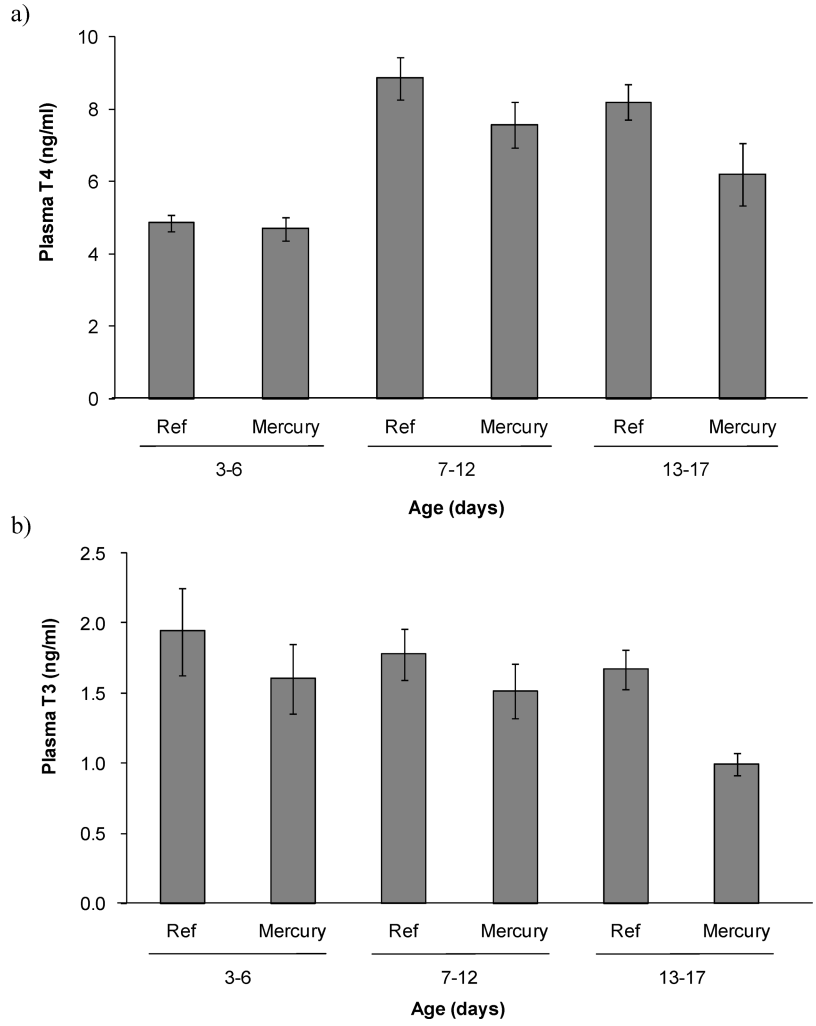

FIGURE 3. Plasma thyroid hormone concentrations were collected from tree swallow (*Tachycineta bicolor*) nestlings in reference (ref) and Hg-contaminated (mercury) sites in Virginia. Figure (a) depicts marginal means of plasma T4 concentrations (ng/mL) ±1 SE in days 3−6, 7−12, and 13−17 nestlings, accounting for the effect of the covariate (time of day). Figure (b) depicts mean plasma T3 concentrations (ng/mL) ±1 SE $n = 10, 10, 11, 12, 13, 12$ for T4 and 10, 10, 12, 12, 13, 12 for T3.

Whereas younger birds in the contaminated sites displayed adrenocortical responses that were nearly twice that of birds from the reference sites, late-stage nestlings (13−17 days old) exhibited pronounced depression of adrenocortical responsiveness at the contaminated sites (Figure 2b). In addition, both plasma T4 and T3 concentrations were lower in the contaminated sites. The site differences in both thyroid hormone levels were most evident in late-stage nestlings. Although we could not examine the relationship between individual blood Hg and hormone concentrations due to the bimodal distribution of Hg concentrations, we propose that the suppression of the HPA and HPT function were due to Hg exposure.

Blood Mercury Concentrations. Birds eliminate a significant portion of their body burden of Hg through deposition into growing feathers, eggs, and feces (*29*−*32*). During nestling and fledgling periods, blood Hg concentrations typically decrease as Hg is deposited into growing feathers (*30*−*32*). Mercury excreted through growing feathers can account for 27% of ingested Hg (*31*) and 26−86% of the total body Hg pool (*25, 30*), indicating deposition of Hg into feathers is an important elimination route for nestlings. However, if Hg intake through diet continues after feathers stop growing or exceeds the rate of elimination to feathers, Hg in blood will rise (*29*−*31*). As in previous studies, nestling swallows in our study decreased blood Hg concentrations as they aged, suggesting Hg was being eliminated into feathers. However, the negative slope of the relationship between age and blood Hg concentrations was significantly steeper in nestlings from the reference sites, suggesting that dietary intake in contaminated sites offset much of the Hg eliminated into growing feathers. As a result, significant accumulation of Hg in target tissues (e.g., brain, liver, and kidney) probably occurred, especially in late-stage nestlings.

Blood Hg concentrations documented at our contaminated study sites were lower than concentrations associated with abnormal behavior or compromised immune function in young birds. Blood Hg concentrations of 1000−2000 ppb ww were not associated with altered immune function, fledging age, or survival in chicks of great egrets, *Ardea albus*, or ospreys, *Pandion haliaetus* (*25, 33*). Similarly, laboratory dosing studies with 100−1500 ppb diet ww, resulting in blood Hg concentrations of 200−3300 ppb ww, did not affect growth, cell-mediated immune response, behavior, or survival in loons (*G. immer*) (*30*). On the other hand, daily administrations of 400 or 500 ppb diet resulted in blood Hg concentrations of 2000 and 12 000 ppb ww, respectively, and caused reduced food intake and lower antibody production in response to sheep red blood cell (*G. immer* and *A. albus*) (*31, 34*). Although these studies indicate that effects of Hg may be species-dependent, blood Hg concentrations observed in our study were well below the suggested threshold for sublethal, adverse effects of Hg in birds (e.g., 400 ppb ww; preliminary lowest observable adverse effect level in loon

chicks, *G. immer* [2]).Using a previous analysis of Hg in the diet of nestlings at our contaminated sites, we estimated the total Hg ingested during the nestling period and compared that cumulative exposure to controlled dosing studies of other species. Adult tree swallows at this study site fed insects that contained on average 970 ppb dry weight (dw) or 330 ppb ww of Hg to their young (*35*) (Brasso, personal communication). Since feeding rates do not change with age of the nestlings or brood size in this species (*36*), we estimated total prey delivered/nestling/day and Hg ingested/body weight. For a brood of six, each nestling received approximately 1004 mg dw prey/day, 974 ppb Hg dw/day, or 344 ppb Hg ww/day (*35*−*37*). This means that day−5, −10, and −15 nestlings at the contaminated sites have ingested approximately 1.72, 3.44, and 5.16 μg Hg ww, or 235, 179, and 238 μg Hg/kg body weight, respectively. In comparison, nestlings at the reference sites had ingested 0.07, 0.15, and 0.22 μg Hg, or 10, 8, and 10 μg Hg/kg body weight at the same time intervals. The cumulative Hg exposure in birds from the contaminated sites is well below that associated with decreased packed cell volume (2300 μg Hg/kg body weight) and food intake (6,400 μg Hg/kg body weight) in piscivorous great egret nestlings (*29, 34*). Additionally, Hg in the nestling diet at the contaminated sites (330 ppb ww) is below that causing reduced antibody production (400 ppb (*38*)) and paralysis (5000 ppb (*39*)) in birds.

Adrenocortical Responses. Differences between swallows from reference and contaminated sites in plasma corticosterone profiles depended on the age of nestlings; adrenocortical responses appeared to be enhanced in contaminated sites in earlier nestling stages, while they were clearly suppressed in the latest stage. This suggests that the duration of Hg exposure and/or nestling age played a role in determining its effect. Similar patterns of enhanced adrenocortical responses followed by suppression have been documented in Hg-exposed fish. For instance, in juvenile rainbow trout (*Oncorhynchus mykiss*), acute exposure to dissolved inorganic or organic Hg elevated plasma cortisol levels within 4 h of exposure, but these returned to baseline by 7 days postexposure (*10*). In contrast, chronic exposure (e.g., 90−180 days) to Hg impaired aspects of the HPA (HPI; hypothalamic-pituitary-interrenal in fish) axis, including reduced plasma cortisol levels as well as enlarged pituitary adrenocorticotropic hormone (ACTH) cells, suggesting an increase in ACTH secretion (*11, 12*).

Cumulative mercury exposure over time may affect the HPA axis in several ways. First, Hg may modify glucocorticoid levels as a result of altered body condition. Body burden of Hg is often negatively correlated with measures of condition (heart mass, fat mass) (*40, 41*) and poor condition can lead to a rise in glucocorticoid levels in birds (*42*−*45*). However, this is unlikely the case in our study because body mass in late-stage nestlings did not differ between sites. Another possibility is that Hg accumulation may surpass a "threshold" of toxicity, altering the HPA axis at the hypothalamus, pituitary, and/or adrenal level. Alternatively, it is possible that suppressive effects of Hg on the HPA axis do not appear until nestlings' adrenocortical responses are fully developed late in the nestling period.

Plasma Thyroid Hormones. Altered thyroid hormone concentrations at the Hg contaminated sites suggest that thyroid hormone production and/or deiodinase activity levels were affected by Hg. Decreased T4 concentrations suggest that thyroid production and/or secretion were low, especially in the late-stage nestlings. Suppressed T3 concentrations could be attributable to reduced T4 production and/or secretion, but the reduction in T3 was more evident than T4 suggesting that other disruptions may also be important. Specifically, the results suggest that 5′-deiodinase activity levels may have been altered in the Hg contaminated sites. Two possibilities are most likely: (1) swallows exposed to Hg could have suppressed liver/kidney 5′-DI which converts T4 to T3, and/or (2) enhanced liver/kidney 5-DI, which converts T3 into inactive T2. Similar relationships between Hg and T3 were found after chronic exposure to Hg in adult humans. Ellingsen et al. (*15*) reported that chloralkali workers exposed to Hg vapors had elevated plasma rT3 levels, suggesting heightened 5-DI activity. On the other hand, liver 5′-DI and plasma T4 (T3 was not measured) appear to be unaffected by 10-day prenatal MeHg treatment in mice, although the methods used were not adequate to determine if there were shifts in T3 and rT3 production (*7*). Effects of Hg on thyroid function have received little attention, and more studies are needed to examine the effects of Hg on thyroid hormone physiology, particularly during periods when thyroid hormones play pivotal roles in life history events (e.g., transition to homeothermy in birds, metamorphosis in amphibians).

The largest difference in thyroid hormone concentrations between sites was observed in the late-stage nestlings. This also suggests that either duration of Hg exposure and/or age of the nestlings influenced the relationships between Hg and the HPT axis. Similar duration-dependent effects of aqueous Hg have been observed in other vertebrates. In rainbow trout, acute exposure to MeHg (4 h) caused elevations of T3 that returned to baseline level within 72 h postexposure (*10*). In contrast, 10-day treatment of $HgCl_2$ decreased both plasma T3 and T4 in mice (*46*). Because it is difficult to compare diverse species, routes of exposure, and forms of Hg, studies examining the effects of Hg on aspects of the HPA and HPT axes (e.g., corticosteroid and thyroid hormone receptor and deiodinase activity levels) are needed to understand the influence of exposure duration and/or age of the young.

Early disruption of endocrine axes can have irreversible effects on birds and other vertebrates. Perinatal alteration of glucocorticoid levels, for instance, permanently modifies how individuals respond to stressful events later in life (*47*). Similarly, hypothyroidism during the pre- and postnatal periods can lead to underdevelopment of the brain, resulting in abnormal behavior, learning abilities, and memory (*13*). Early exposure to Hg may permanently alter HPA and HPT axes, thus reducing the probability of survival later in life. Further research is needed to investigate the direct causal relationship between Hg and endocrine disruption and the latent effects of pre- and postnatal endocrine disruption by Hg in vertebrates.

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Supporting Information Available

A map of study sites and details of the hormone assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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