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FEATHER GROWTH INFLUENCES BLOOD MERCURY LEVEL OF YOUNG SONGBIRDS

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Abstract—Dynamics of mercury in feathers and blood of free-living songbirds is poorly understood. Nestling eastern bluebirds (*Sialia sialis*) living along the mercury-contaminated South River (Virginia, USA) had blood mercury levels an order of magnitude lower than their parents (nestling: 0.09 ± 0.06 mg/kg [mean ± standard deviation], $n = 156$; adult: 1.21 ± 0.57 mg/kg, $n = 86$). To test whether this low blood mercury was the result of mercury sequestration in rapidly growing feathers, we repeatedly sampled free-living juveniles throughout the period of feather growth and molt. Mean blood mercury concentrations increased to 0.52 ± 0.36 mg/kg ($n = 44$) after the completion of feather growth. Some individuals had reached adult blood mercury levels within three months of leaving the nest, but levels dropped to 0.20 ± 0.09 mg/kg ($n = 11$) once the autumn molt had begun. Most studies of mercury contamination in juvenile birds have focused on recently hatched young with thousands of rapidly growing feathers. However, the highest risk period for mercury intoxication in young birds may be during the vulnerable period after fledging, when feathers no longer serve as a buffer against dietary mercury. We found that nestling blood mercury levels were not indicative of the extent of contamination because a large portion of the ingested mercury ended up in feathers. The present study demonstrates unequivocally that in songbirds blood mercury level is influenced strongly by the growth and molt of feathers.

Keywords—Bird Feather Fledgling Mercury Molt

INTRODUCTION

Mercury from anthropogenic sources has contaminated water bodies worldwide. Bioavailable methylmercury can enter aquatic food chains and biomagnify to levels of concern in top predators [1]. Methylmercury contamination can have deleterious effects in fish-eating birds, including on behavior, reproductive success, and survival [2,3]. Elevated levels of mercury have also been found in terrestrial-feeding songbirds [4–6], though few studies have demonstrated adverse effects levels of mercury in free-living birds of any species (but see Brasso and Cristol [7] and Evers et al. [8]). Research is needed to determine if mercury is negatively impacting populations of birds, including songbirds, as well as to identify the life stages of highest risk.

Birds appear to reduce their body burden of mercury and mitigate toxicity through several mechanisms, including feather growth. Methylmercury has a high affinity for free thiol groups (-SH) that are abundant in feather keratin [9,10]. When feathers grow they are connected to the body by a blood vessel; circulating mercury can then be transported to the feather and incorporated into the keratin structure [10]. When feather growth is complete, shortly after fledging in young songbirds or after each molt in adults, the feather is no longer supplied with blood and the mercury remains physically and chemically stable within the feather [10,11].

The common assumption that growing feathers serve as an elimination route for mercury has been supported primarily by indirect evidence and entirely with data from fish-eating and aquatic birds. It is widely stated in the literature that a large proportion (50–93%) of the body burden of mercury is found in feathers [12–15]. Typically, researchers have sampled dead seabirds or wading birds at different ages or molt stages to describe the changing body burden of mercury. One of the first studies [16] reported lower mercury levels in later-grown feathers, suggesting that mercury body burden had been reduced by earlier feather growth. Others have reported increasing body tissue mercury level with age of nestlings, coincident with the completion of feather growth [12]. Further, tissue mercury levels decreased during the growth of feathers in molting adults of several species [13,17]. Laboratory dosing studies have corroborated these findings by documenting faster turnover of blood mercury during feather growth [18–20]. The few existing field studies on free-living birds have shown an increase in blood mercury levels in later growth stages of nestling birds ([21,22]; but see Caldwell et al. [23]), although none have examined the period after young birds leave the nest, when feather growth ceases altogether.

Our objective was to examine mercury levels directly in the blood of free-living young songbirds on a mercury-contaminated site that were receiving a mercury dose in their natural diet. We predicted that blood mercury levels would be related to the degree of feather growth and would rise to adult levels soon after feather growth ceased. We used radio telemetry to locate eastern bluebirds (*Sialia sialis*) after they fledged from nests. Individual birds were retrapped repeatedly and blood and feather mercury was measured with each capture. We assumed that the dose of mercury was relatively constant in the diet across the period of the study. To test this assumption we collected and examined mercury levels of prey items fed to nestlings at the site throughout the season. As an alternative test of whether changes in blood mercury level might reflect

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shifts in diet, we estimated fledglings' trophic levels through analysis of stable isotopes of nitrogen.

The partitioning of contaminants to different wildlife tissues is of interest both in designing effective monitoring regimes and predicting injury. Nestling blood has been used as a biomarker because it contains contaminants that are localized spatially and temporally, and nestlings are relatively easy to sample compared to other life stages [24]. The present study examined the appropriateness of nestling blood as a monitoring tissue. In addition, by tracking post-fledging birds we investigated whether they experience a surge in blood mercury at a time of great vulnerability, as they become independent and face heightened mortality from predation, disease, and starvation [25].

MATERIALS AND METHODS

Study site

Mercuric sulfate, a catalyst in the manufacture of synthetic fibers, entered the South River from an industrial plant in Waynesboro, Virginia, USA, between 1929 and 1950 [26]. More than a half century later, mercury is elevated in fish (http://www.deq.virginia.gov/fishtissue/mercury.html) and birds compared with natural background levels [6]. The contaminated study area encompassed a 38.6-km portion of the South River from the contamination source (38°3′49″N, 78°53′5″W) downstream to the confluence with the North River (38°17′45″N, 78°48′29″W, Fig. 1). Fledglings were monitored at and around five locations in Virginia: Waynesboro's water treatment facility; Basic Park, a developed recreational park; hay fields near the former Genicom Corporation industrial site; Augusta Forestry Center, a state forestry nursery; and Grottoes City Park, a mixed-use town park in Grottoes (Fig. 1). Fewer samples were required in 2007, therefore only Augusta Forestry Center, the site with the highest concentration of nesting bluebirds, was used as a monitoring location. Sites for monitoring fledglings were chosen based on accessibility for radio tracking and mist-netting, including on adjacent properties.

Field methods

Over 200 nest boxes were monitored in grassland and forested habitat within 50 m of the river as part of related studies [6,7]. In 2006, bluebird nestlings were banded when they were between 15 and 17 d old with a U.S. Geological Survey numbered leg band and a unique combination of three colored plastic bands. To assist in locating birds after they had fledged from the nest box, radio transmitters (n = 46; 0.9-g model BD-2 transmitters, Holohil Systems, Ottawa, ON, Canada) were attached to two to five nestlings within each brood. In 2007, nestlings were again banded, but transmitters were not needed to locate them as fledglings.

Transmitters were attached using a Rappole harness constructed of a combination of 1-mm elastic bead cord glued in a figure-eight shape to the transmitter with cyanoacrylate glue, as well as loops of 0.5-mm elastic cord threaded through the existing hollow tubing at the front and back end of the transmitter [27]. The weight of the transmitter plus harness was 1.1 g, which was below a published guideline for small birds of 5% of body weight (nestling mass ranged from 23.5 to 32.6 g) [28]. We tracked birds on foot at each site using handheld receivers (model R-1000, 149–152 MHz, Communications Specialists, Orange, CA, USA) and handheld folding Yagi three-element directional antennas (model F 151-3FB, AF Antronics, Urbana, IL, USA).

Once relocated, fledglings were observed until a typical pattern of movement was identified. Trapping attempts began approximately two weeks after fledging. Mist nets were set up in the area where fledglings were located, always <400 m from the river. Bluebird broods typically fledge synchronously and juveniles form cohesive flocks with other families, often remaining together in the natal area throughout the summer and into the fall [29]. This social behavior facilitated trapping and also increased the likelihood that fledglings continued to feed in the contaminated area.

Morphological measurements (weight, wing chord, and tail length) were taken on nestlings and with each recapture of fledglings. Individual growing feathers, both flight and body, were counted or classified as >100. Before analysis, we defined four sequential feather growth categories as follows: *nestling*, when thousands of feathers were growing simultaneously (<17 d); *waning*, just after fledging when feather growth was decreasing but >10 feathers were still growing (27–41 d); *none*, when feather growth had ceased (32–80 d); and *molt*, when >10 body feathers were growing to replace juvenile plumage (43–106 d).

Blood and feather samples were taken from nestlings and fledglings in both years. A 26-gauge needle was used to puncture the cutaneous ulnar (or brachial) vein, and two half-filled 75-μl heparinized capillary tubes of blood were collected from each individual. Feathers were sampled from the belly (n = 6–9) and back/rump (n = 6–9). Blood and feather samples were placed on ice in the field and then stored in a freezer (−25°C) until analysis.

Prey items were collected from several songbird species breeding on site throughout the season in 2006 using the ligature method [30]. A small cable tie was placed around the necks of each nestling in broods of eastern bluebirds, Carolina wrens (*Thryothorus ludovicianus*), or house wrens (*Troglodytes aedon*) and left in place during parental feeding. The ligature was tight enough to prevent prey items from being swallowed, but loose enough to allow breathing. After 1 h, prey items were collected from the mouths of the nestlings and stored in a freezer prior to analysis.

Laboratory analysis—mercury

To remove external particulate contamination before analysis, feathers were washed with deionized water and dried in a low-humidity chamber for approximately 48 h. Prey items were freeze-dried using a Labconco® Benchtop Freeze Dry System (Kansas City, MO, USA) for 24 to 48 h, then homogenized prior to analysis. In 2006, samples were analyzed for total mercury at the Trace Element Research Laboratory (Texas A&M University, College Station, TX, USA). In 2007, all samples were analyzed at the College of William & Mary, including a few remaining samples from 2006. Samples were analyzed for total mercury, which approximates the amount of methylmercury; 90 to 100% of mercury in avian blood and feathers is methylmercury [5].

At both labs, blood, feathers, and prey items were analyzed by cold vapor atomic absorption spectroscopy using a Milestone® DMA-80 direct mercury analyzer (Shelton, CT, USA). Minimum detection limit was 0.0051 to 0.0055 mg/kg. A sample blank, methods blank, duplicate sample, and two of three standard reference materials (DORM-2, DORM-3, or DOLT-3) were run every 20 samples. Recovery of total mercury was

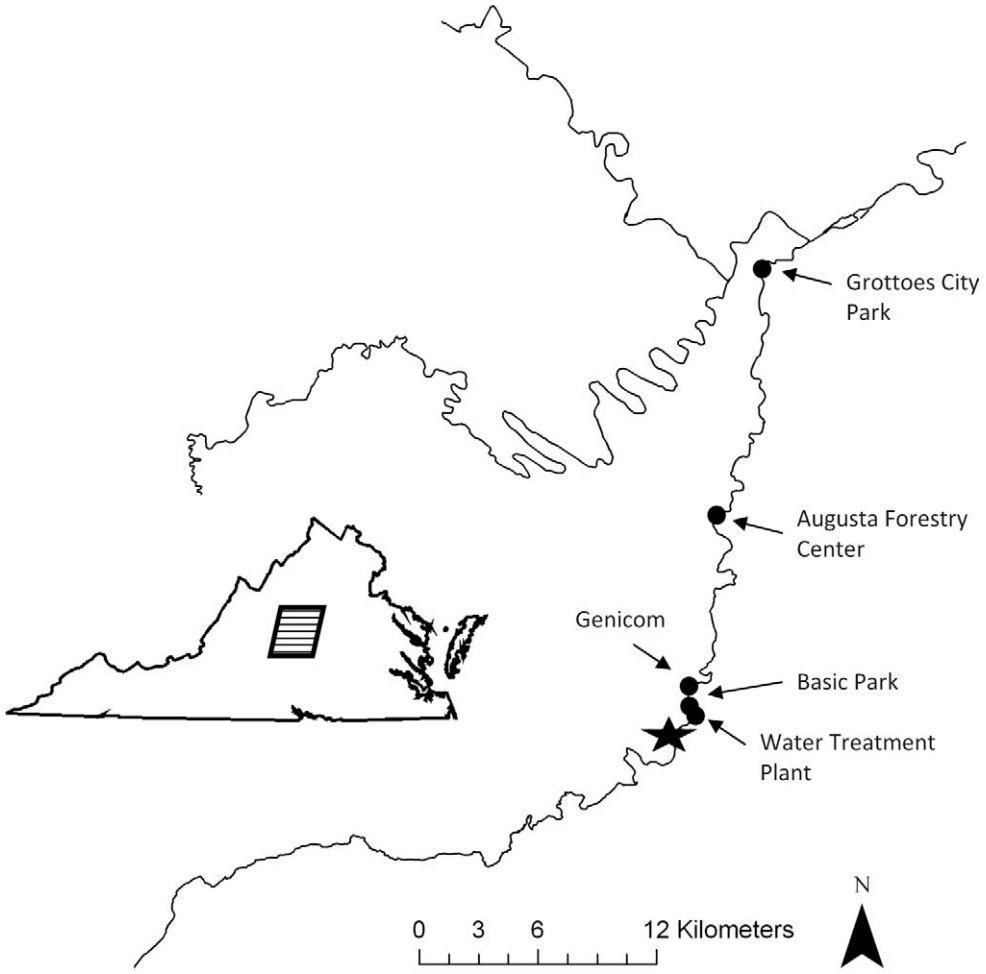

Fig. 1. Study sites along the South River, Virginia, USA, are represented by black dots. Striped box represents location of the river within Virginia. Star indicates the location of the original source of contamination on the South River.

96 to 99% for all three standards. Duplicate samples were obtained by splitting the total number of feathers in half, dividing a homogenized invertebrate, or analyzing two capillary tubes of blood from the same collection of the same bird. Interlaboratory duplicates were also run, to ensure comparability between the two labs. The relative percent difference not used again between interlaboratory duplicates was 15.73 ± 27.53% for samples greater than 10 times the minimum detection limit, less than the generally accepted 20%. Mercury levels are reported as milligrams per kilogram wet weight for blood and prey items or fresh weight for feathers.

Laboratory analysis—stable isotopes

Blood for isotope analysis was collected simultaneous with mercury samples, but stored in a nonheparinized capillary tube. Samples were then freeze-dried and shipped to the University of California-Davis Stable Isotope Facility (Davis, CA, USA) for analysis. Ratios of stable isotopes of nitrogen were measured by continuous-flow isotope ratio mass spectrometry (20-20 mass spectrometer, Sercon, Crewe, UK). The samples were combusted to N_2 at 1,000°C in an on-line elemental analyzer (PDZEuropa, Sandbach, UK; ANCA-GSL, Cheshire, UK). Sample ratios were compared to those of pure cylinder gases injected into the spectrometer before and after the sample peaks. Stable isotope ratios are reported in parts per thousand (‰), in the standard delta (δ) notation, of the standard for nitrogen (atmospheric nitrogen, AIR). The equation, $\delta X = [(R_{sample}/R_{standard}) - 1] \cdot 100$, was used to calculate values, X is the heavier isotope, ^{15}N; R_{sample} is the isotopic ratio in the sample; and $R_{standard}$ is the ratio in the standard [31]. Measurement errors averaged ± 0.1‰.

Statistical analyses

Statistical tests were performed using Minitab® 15 (Minitab® version 15, State College, PA, USA) or R 2.5.1 (version 2.5.1, R Development Core Team, Vienna, Austria). Nonnormal data were log-transformed, or nonparametric tests were used as noted. A significance level of $\alpha < 0.05$ was used for all tests. If an individual was caught twice during the same feather growth stage, values were averaged (for mercury level, date, age, and morphological measurements). We used repeated measurements of the same individuals over time to directly monitor changes in mercury and $\delta^{15}N$ using a linear mixed-effects model fit by maximum likelihood estimates. Factors in the model included: distance from the source of contamination and feather growth stage or age. Distance from the source of contamination was included as a factor to ensure that mercury levels were not driven by environmental mercury availability. Factors used in earlier versions of the model for blood mercury (distance between point of capture and the river, sex, and nitrogen isotope ratio) were all nonsignificant and thus elimi-

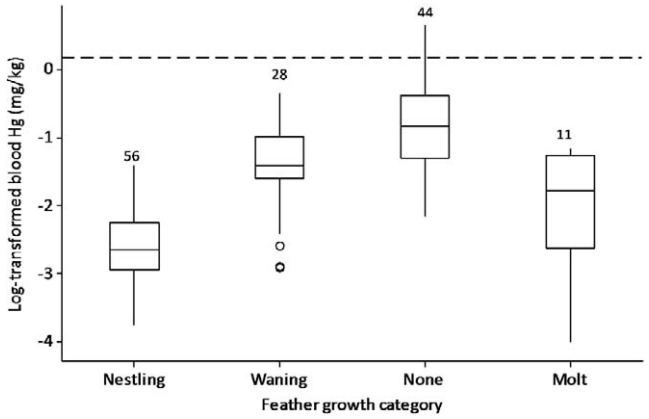

Fig. 2. Fledgling bluebird blood mercury (log-transformed, mg/kg) in the four chronological feather growth stages. Samples sizes are above the bars. Dashed line indicates mean adult blood mercury level.

nated. To test for changes in prey mercury level across the season in 2006, we used regression analysis.

RESULTS

In 2006, 46 individuals banded as nestlings were caught after fledging, of which 20 did not have transmitters attached in the nest box. Individuals were caught from the first (n = 31) and second (n = 15) broods, belonging to 12 families. In 2007, 12 banded fledglings were caught, nine individuals originating from the first brood, and three from the second. Combining years, a total of 56 birds were sampled during the nestling period, 28 as feather growth was waning, 44 with feather growth classified as none, and 11 during molt.

Blood mercury levels

On the contaminated site, mean nestling blood mercury levels were 0.09 ± 0.06 mg/kg (n = 156), significantly lower than adult levels (mean = 1.21 ± 0.57 mg/kg; n = 86; T = 34.29, df = 195, $p < 0.001$). Feather growth stage had a significant effect on fledgling blood mercury, which increased until the none growth stage and then decreased during molt ($y = -1.24 + 0.59x - 1.12x^2$, $p < 0.001$; Fig. 2). There was no significant effect of distance from the source (river km) on blood mercury (linear slope [lin]: −0.026, p = 0.06). The relationship of feather growth to blood mercury was alternately examined in the context of age as opposed to growth stage. Log-transformed blood mercury had a similarly significant quadratic relationship with age ($y = -1.32 + 6.91x - 5.24x^2$, $p < 0.001$; Fig. 3) and no significant relationship with river km (lin: −0.026, p = 0.10).

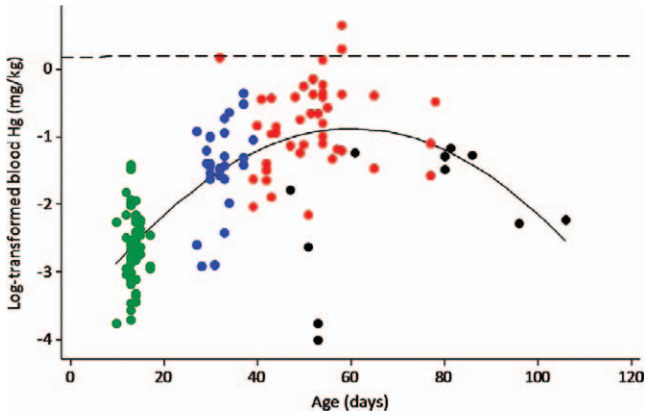

Fig. 3. Blood mercury (log-transformed, mg/kg) plotted against age of fledgling. Colors represent different feather growth stages: Green represents nestling; blue represents waning; red represents none; black represents molt. Black circles (n = 5) <65 d old represent birds from the second clutch that began molt at a younger age than birds from the first clutch. Dashed line represents mean adult blood mercury level.

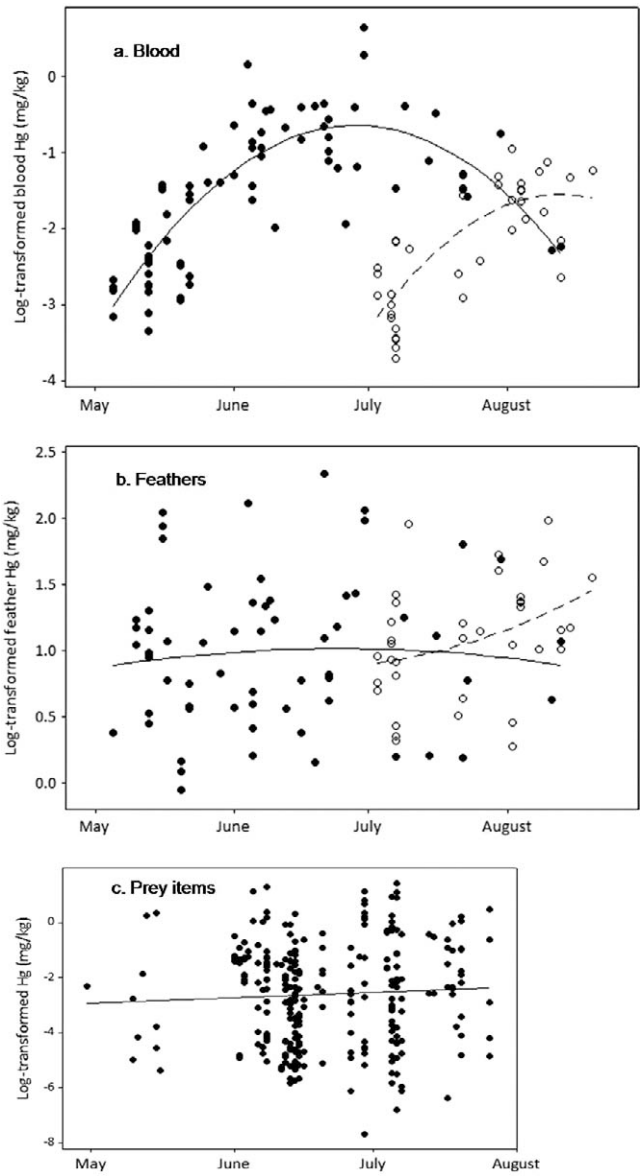

Fig. 4. Blood mercury levels (**a**; log-transformed, mg/kg), feather mercury levels (**b**; log-transformed, mg/kg), and prey mercury levels (**c**; log-transformed, mg/kg) over the season in 2006 only. In panels **a** and **b**, closed circles represent birds from the first clutch and open circles represent birds from the second clutch.

Because bluebirds bred twice during each season, we conducted separate analyses for the blood from the first and second clutches (Fig. 4a). Birds from the first clutch exhibited a significant relationship between blood mercury level and both feather growth stage ($y = -1.04 + 0.76x - 1.15x^2$, $p < 0.001$) and river km (lin: −0.021, p = 0.03). Blood mercury from second clutch birds also had a significant relationship with feather growth stage ($y = -2.16 + 0.35x - 0.92x^2$, $p < 0.001$), but not with river km (lin: −0.01, p = 0.71).

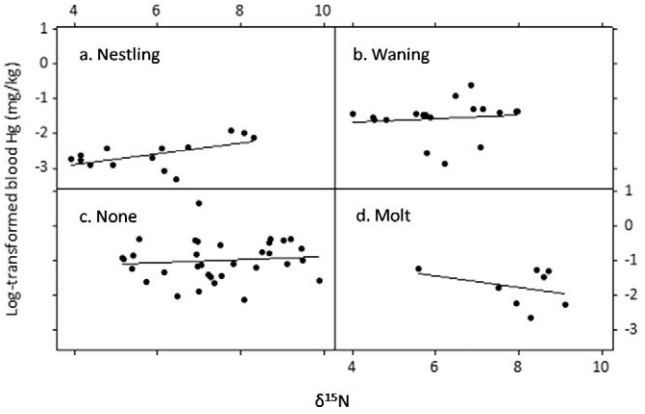

Fig. 5. $\delta^{15}N$ and blood mercury levels (log-transformed, mg/kg) in each stage of feather growth: (**a**) nestling, (**b**) waning ($F_{1,16} = 0.26$, $p = 0.63$, $R^2 = 0.01$), (**c**) none ($F_{1,31} = 0.39$, $p = 0.54$, $R^2 = 0.012$), (**d**) molt ($F_{1,6} = 0.72$, $p = 0.43$, $R^2 = 0.10$).

Feather mercury levels

The same terms (river km and feather growth stage) were used in analyzing feather mercury (Fig. 4b). Feather growth stage had a weak positive effect on feather mercury ($y = 1.64 + 0.18x$, $p = 0.002$), while river km had a negative effect (lin: -0.316, $p = 0.002$). Alternately, feather mercury also had a significant weak positive relationship with age ($y = 1.61 + 0.76x - 0.75x^2$, $p = 0.001$; Fig. 4b) and negative relationship with river km (lin: -0.032, $p = 0.0015$).

Prey mercury across the season

Mercury levels of prey items (Fig. 4c) in 2006 did not change over time ($p = 0.362$, $R^2 = 0.03$) or with river km ($p = 0.454$; $R^2 = 0.02$). Prey groups represented included Araneae (spiders; $n = 20$), Coleoptera (beetles; $n = 12$), Lepidoptera (moths and butterflies; $n = 25$), Orthoptera (crickets and grasshoppers; $n = 11$), and unidentified invertebrates and fruits ($n = 25$).

Stable isotopes of nitrogen

Isotopic ratios of nitrogen sampled from a subset of nestlings and fledglings ($n = 68$) were significantly positively related to feather growth stage ($y = 8.49 + 1.54x$, $p < 0.001$). River km had a negative linear effect on $\delta^{15}N$ values (lin: -0.06, $p = 0.046$).

Blood mercury levels and $\delta^{15}N$ were analyzed separately because they each have independent relationships with feather growth. To examine the relationship of blood mercury to $\delta^{15}N$ signature, regression analysis was used within each feather growth category (so no individuals were repeated). Within each feather growth category $\delta^{15}N$ did not correlate with blood mercury except during the nestling stage ($F_{1,12} = 6.36$, $p = 0.027$, $R^2 = 0.35$; Fig. 5).

DISCUSSION

Fledgling blood mercury

As predicted, a window of mercury elimination was provided when young nestlings grew thousands of feathers in less than three weeks. Shortly after fledging, however, this route of elimination was no longer available and blood mercury levels rose nearly to adult levels (see Fig. 3). Blood mercury levels fell once molt began and this elimination route reopened, confirming the influence of feather growth on blood mercury. Blood mercury levels exhibited a similar pattern when analyzed by age because feather growth stage corresponded closely with age (see Fig. 3). That feather growth stage is a better predictor of blood mercury than age is evident when comparing patterns of change in mercury level between the two clutches, which had different timing of molt (see Fig. 4a). Birds from both broods exhibited the same decline in blood mercury with the onset of molt, despite the fact that birds from the second brood molted at a younger age (see Fig. 3).

Existing research on mercury levels and plumage overwhelmingly agrees with our findings, despite differences in approach. Past studies on fish-eating, wading, and oceanic birds have documented that 50 to 93% of body mercury burden and 42 to 60% of ingested mercury is incorporated into the feathers [12,14,15,18–20]. However, these studies did not account for wide feeding ranges and differences in mercury intake among species sampled. They also did not track changes within individuals or examine the relationship between blood and feathers throughout the vulnerable post-fledging period.

Young bluebirds, which remained on or near a natal contaminated site throughout the late summer and fall, are likely receiving repeated small doses of mercury on a regular basis. In past dosing studies, mercury levels were monitored after a single dose was administered, relying on the assumption that the kinetics would be the same as for constant dietary intake [18–20]. Although the present study provided no information on excretion rate or half-life of mercury in the blood, blood mercury levels decreased during periods of maximum feather growth, consistent with laboratory studies; a phenomenon already documented under controlled circumstances has now been found to occur in free-living populations.

One objective of the present study was to determine whether findings from previous studies of large, aquatic, fish-eating birds may be generalized to songbirds. Songbirds comprise the majority of all bird species, but until recently they have been largely overlooked by toxicologists because few eat fish. However, there is increasing concern that these species may be exposed to harmful mercury levels [6]. Previously studied species may differ from songbirds in molting patterns, metabolism, and possibly kinetics of mercury in the body. In fact, they may have been under selective pressure for millennia to eliminate dietary mercury, which has always been present in marine or piscivorous food webs. This is the first demonstration that the oft-cited phenomenon of elimination into plumage occurs in small birds, in terrestrial birds, and in free-living birds that were followed as individuals.

Other factors could have influenced blood mercury levels. Location (referred to as river km) was sometimes a significant effect in analyses of blood mercury level; however, this merely reflected the highly variable mercury exposure at different sites and did not affect changes in blood mercury within sedentary individuals across the study period. There may also be variation between individuals or the sexes in kinetics of mercury in the body (see Bearhop et al. [32]). However, sex was not a significant factor in the variation of mercury levels of fledgling bluebirds, and any genetic (familial) differences were also accounted for in the analysis. Another possible mechanism for changing mercury in the body is growth and protein turnover; as muscles grow, mercury concentrations are diluted [33]. Growth dilution may have been partly responsible for keeping blood mercury levels low in growing nestlings; however, this does not diminish the importance of feather growth in mercury

elimination because molting fledglings that were no longer growing experienced a similar reduction in mercury.

Another consideration is that fledglings with the lowest blood mercury might have been present later in the season not because of feather molt, but because those with higher mercury had already died from mercury intoxication. However, there was no obvious pattern of mortality related to initial mercury level, suggesting that probability of survival to the end of the study was not affected by nestling mercury level. Of the 11 birds that were caught as molting fledglings, approximately half ($n = 5$) had started as nestlings with blood mercury levels above the mean. In fact, the direction of change in mercury level between feather growth stages within individuals was as predicted 97% of the time (76/78), regardless of an individual's initial mercury level.

Fledgling feather mercury

There was a statistically significant increase in feather mercury with both feather growth stage and age but it was biologically insignificant, perhaps driven by the sample of birds from the second clutch (see Fig. 4b). Even this small increase was unexpected, however, as mercury in feathers is stable after growth is complete, and only fully grown feathers were sampled from fledgling birds [11]. The increase was likely due to the fact that nestling feathers were still growing when sampled and thus additional mercury may have been deposited until growth was complete. Mercury may have been added to the outside of feathers, via the preen gland oil or contaminated dust, as birds aged [34]. Because feathers were washed only in deionized water, some exogenous mercury may have adhered to feathers. Regardless of the explanation, feathers increased very little in mercury content, as expected, during the same time period in which blood mercury rose and then fell.

Did change in diet influence change in blood mercury?

We assumed that dietary intake of mercury was constant across the season, and thus did not explain changes in mercury level. Our most direct test of this assumption supported it; prey mercury content did not change throughout the season. However, the prey we sampled were gathered by adult birds of three songbird species and fed to nestlings, and thus may not have been identical to the diet of fledgling bluebirds.

To test for a seasonal shift in fledgling diet we sampled stable isotopes of nitrogen from fledgling bluebird blood and found a seasonal increase. This change in ratio of nitrogen isotopes could indicate a shift in diet towards prey higher in the food chain, which could have contributed to the increase in fledgling blood mercury during the waning period. However, the increase in ratio of $\delta^{15}N$ continued through the molt period, while blood mercury levels dropped. It is possible that the increase in $\delta^{15}N$ indicated a true diet shift, but if so, the diet shift was not closely tied to blood mercury levels. Individual diets were not related to individual mercury levels, as documented by the lack of correspondence between mercury level and isotopic signature within each stage of feather development. Apparently there was a decoupling of mercury level and stable isotope ratio, and individuals with the highest mercury were not generally those with the highest $\delta^{15}N$ (see Fig. 5). Even if the dietary shift was towards prey items higher in the food chain, and thus presumably with more methylmercury content, young bluebirds still substantially reduced blood mercury levels through growth of feathers.

The reason for the increase in $\delta^{15}N$ with age is unclear and could be the result of a seasonal change in isotopic signature of the prey base. Bluebird prey (fed to nestlings) on the South River consisted primarily (>75% of biomass) of Aranea, Lepidoptera, Orthoptera, and Coleoptera (S. Friedman, Master's thesis, The College of William and Mary, Williamsburg, VA, USA). The availability of certain insects may have changed throughout the summer; anecdotally, there were short-term increases of abundance of June bugs (*Phyllophaga* spp.) and grasshoppers (family: Acridinae). Bluebirds may have shifted their diets depending on what was available, thus changing their nitrogen signature, but this did not correspond with temporal changes in blood mercury.

Other speculative explanations exist for why $\delta^{15}N$ might increase without an actual change in trophic level. Past studies present conflicting evidence on whether nitrogen isotopes increase with age or body size [35–37] or with nutritional stress [38] (but see Kempster et al. [39]). It will require a controlled laboratory study monitoring $\delta^{15}N$ over time in birds on a known diet to untangle the relationship of isotopic signature with age and growth.

Implications

Although young birds may be more sensitive to contaminants—for example intestinal absorption of heavy metals is enhanced in very young organisms [40]—it is likely that they are buffered from mercury toxicity by growing feathers. Only after feather growth is completed, usually shortly before becoming independent, are they susceptible to accumulating high concentrations of mercury. Feather growth predicting blood mercury level should be included in any risk analysis for a bird species with a known molt schedule.

Risk of predation in some species is high during the first week after fledging, and again when juveniles are no longer attended by the parents [25]. For songbirds living on mercury-contaminated sites, this high-risk period will usually correspond with increases in blood mercury. Rapid increases in accumulation of mercury in internal tissues may have neurological and behavioral effects, possibly causing birds to be more susceptible to predation or less likely to acquire important survival skills. Juvenile survival is one of the main factors considered in population demographic studies, and the possibility of a surge in contaminants in recently fledged birds should be considered.

In the present study, the duration of the surge in blood mercury was greater for birds originating from the first clutch. Second clutch fledglings began molting at an earlier age to catch up before the onset of winter. Therefore, fledglings from the first clutch had a longer period of no feather growth, by approximately 20 d, than fledglings from the second clutch, and experienced elevated mercury in blood for approximately twice as long before being rescued temporarily by feather molt. Fledglings from the first clutches at mercury-contaminated sites could experience higher mortality than birds from second clutches due to this temporal difference in molt schedule, but further study will be needed to test this hypothesis.

Past studies have established a strong foundation of evidence that mercury in the body is preferentially bound into feathers as they grow. Recent studies show increasing evidence that methylmercury accumulation is occurring in terrestrial systems and affecting insectivorous passerines [4–7]. The present study has important implications for experimental design of mercury studies and the use of nestlings as bioindicators. If only blood in nestling birds is monitored, the pres-

ence of mercury and evaluation of risk may be severely underestimated. Eventual effects of mercury on growth or survival of young birds might be delayed until they are no longer buffered from toxicity by feather growth. It is important to continue monitoring birds during the post-fledgling period and outside of molts to comprehensively assess the risks to survival.

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