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Mercury exposure of a wetland songbird, *Agelaius phoeniceus*, in the New York metropolitan area and its effect on nestling growth rate

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Abstract The potential for mercury accumulation in free-living passerine birds is now recognized to be much greater than previously assumed. However, lowest observable effect levels have yet to be well established for this taxonomic group and it is usually unknown whether levels observed in the wild are causing adverse effects. We measured total blood mercury (THg) levels and took repeated morphological measurements from nestling red-winged blackbirds (*Agelaius phoeniceus*; N=39) in the New York metropolitan area to investigate whether mercury affected their growth rate. We also compared THg levels of nestlings (and parents; N=14) between our two study sites, which included riparian habitats along a city river and surrounding ponds in a nearby suburb, to examine differences between birds within and beyond the urban core. THg levels ranged 0.009–0.284 ppm in nestlings and 0.036–0.746 ppm in adults. Adults and nestlings had significantly higher THg outside of the city than within, possibly due to the ability of rivers to flush contaminants and the higher methylation potential of ponds. Among our candidate sets, models containing THg had minimal support for explaining variation in nestling growth rate. Summed Akaike weights further showed that THg had little relative importance. Mercury pollution in our sites may be low, or feather growth may have been sufficient to protect nestlings from accumulating harmful mercury levels in living tissues.

Keywords Blood mercury · Red-winged blackbird · Growth rate · New York City · Urban habitat

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Introduction

Mercury accumulation has been well documented in piscivorous birds and raptors, and has been shown to cause adverse effects such as reduced egg production, poor hatching success, and aberrant parental care. In contrast, groups of birds at lower trophic positions, such as songbirds (order Passeriformes), have traditionally been overlooked and considered to be at relatively low risk for mercury exposure (Seewagen 2010). Recent findings of detectable mercury concentrations in several species of insectivorous songbirds are suggesting that methylation in terrestrial systems and biomagnification through invertebrate communities may be much greater than previously expected. Mercury concentrations comparable to or exceeding those observed in piscivorous

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waterbirds have been recorded in insectivorous songbirds with diets of aquatic origin (e.g., Evers et al. 2005; Brasso and Cristol 2008; Cristol et al. 2008; Tsipoura et al. 2008). Even insectivorous or omnivorous forest songbirds with diets from strictly terrestrial food webs can be highly exposed to mercury (Cristol et al. 2008; Rimmer et al. 2005; Rimmer et al. 2010).

Although mercury accumulation in songbirds has begun to receive increased attention and songbird mercury levels are being more commonly measured, it is seldom clear whether the observed levels are great enough to cause sublethal adverse effects (e.g., Rimmer et al. 2005; Rimmer et al. 2010; Edmonds et al. 2010). So far, few field or laboratory studies have directly related mercury to the development, behavior, health, or fitness of songbirds, making it difficult to interpret the significance of these measurements (Seewagen 2010). Relative to songbirds inhabiting reference sites, those inhabiting areas with heavy point-source mercury contamination have been found to have compromised endocrine function (Wada et al. 2009), impaired immunocompetence (Hawley et al. 2009), abnormal song structure (Hallinger et al. 2010), skewed sex ratios (Bouland et al. 2012), and lowered hatching and fledging success (Brasso and Cristol 2008), but these studies have not identified threshold levels at which such effects begin to appear. Jackson et al. (2011) recently established the first threshold effect level for reduced reproductive success in a wild songbird, the Carolina wren (*Thryothorus ludovicianus*), but despite this important advance, our understanding of how environmental mercury is affecting wild songbirds remains limited, particularly in places without substantial point-source mercury contamination.

We measured blood mercury levels in adult and nestling red-winged blackbirds and tested the relationship between nestling mercury level and growth rate to investigate whether mercury accumulation in this songbird species was hindering development. Mercury has been found to depress growth or body mass in several groups of birds, such as domestic poultry, raptors, and waterbirds (e.g., Fimreite and Karstad 1971; Grissom and Thaxton 1985; Spalding et al. 2000; but see Kenow et al. 2003), but this effect has yet to be naturally or experimentally observed in songbirds, to our knowledge. Some mechanisms by which mercury could reduce nestling growth rate include decreased appetite of, and food consumption by, nestlings, and aberrant incubation and provisioning behavior by mercury-exposed parents (Spalding et al. 2000; Kenow et al. 2003; Evers et al. 2008). We conducted our study at two sites, one urban and one suburban, in the New York metropolitan area and compared blood mercury levels of birds between them. We expected to find a negative relationship between blood mercury level and nestling growth rate and greater blood mercury levels in birds within New York City than in those outside of the city because of the urban study site's long history of industry and various forms of pollution.

Methods

Study species and sites

The red-winged blackbird is a common and widespread passerine that nests in wetland and riparian habitats throughout North America. Mercury concentrations exceeding those associated with piscivorous waterbirds have been reported in red-winged blackbirds, suggesting that this species may be at a particularly high risk of mercury exposure (Evers et al. 2005; Tsipoura et al. 2008). Red-winged blackbirds feed nestlings mostly emerging aquatic insects and terrestrial arthropods (Yasukawa and Searcy 1995), both of which can accumulate and expose their predators to methylmercury (Longcore et al. 2007; Brasso and Cristol 2008; Cristol et al. 2008).

We studied red-winged blackbirds nesting along a reach of the Bronx River that passes through Bronx Park in New York City, NY (40°51′ N, 73°52′ W), and at Kenridge Farm in Cornwall, NY (41°25′ N, 74°02′ W). The Bronx River begins at Davis Brook in Valhalla, NY and runs through the Bronx before joining the East River. The river has a long history of pollution from urban and former industrial sources (Hoellein et al. 2011). Coal-powered trains followed the river corridor for much of the 1800 s and may have been a local source of mercury input in the past. We collected data from nests found along the river's edge and on its small, vegetated islands.

Kenridge Farm is approximately 80 km northwest of New York City, in a suburban area of the Hudson Highlands region. The 72-ha property was pastureland in the 1700 s and was then converted to cropland in the early 1800 s. It is presently managed as grassland habitat for wildlife. The site has permanent and ephemeral ponds that are maintained through snowmelt, rainfall, and groundwater inputs. We studied red-winged blackbirds nesting in the vegetated margins of these ponds and in the surrounding floodplains.

Field data collection and blood sampling

Fieldwork was conducted along the Bronx River from 13 May to 3 July, and at Kenridge Farm from 29 May to 29 July, 2010. We searched for and monitored nests at each site every other day, following Martin et al. (Martin et al. 1997). After locating a nest, it was numbered and mapped, and a pink flag was placed about 5 m away to aid relocation.

We measured nestling body mass to 0.1 g with a digital balance, and tarsus length and wing chord to 1 mm with digital calipers. Nestlings were measured at least four times between the ages of 0 and 11 days. Nestlings <8 days old were marked on both tarsi with a unique combination of black, green, and blue nontoxic marker. We banded nestlings with USGS aluminum leg bands at 9 days of age. We estimated age based on physical features such as feather pin size and degree of eye opening if the exact hatching date of a nestling was unknown (Holcomb and Twiest 1971). Handling of nestlings was brief and was not expected to influence their rates of growth (Gebhardt-Henrich and van Noordwijk 1991; Butler and Dufty 2007). We also captured adults that were observed attending to the monitored nests using mist nets and playback recordings. Adults were banded, sexed (based on plumage), weighed, and measured (wing chord).

Blood samples were collected from adults and nestlings and used to indicate the degree of recent mercury exposure from local food sources (Evers et al. 2005). We took samples from adults and 8–9-day-old nestlings by medial metatarsal or cutaneous ulnar venipuncture using a 26 gauge needle and heparinized capillary tube. No more than 10 % of total blood volume was collected (Fair et al. 2010). Samples were stored in the field in a cooler with ice packs and then transferred to a −20 °C freezer at the end of the day.

Mercury analysis

Blood samples were analyzed at the College of William and Mary (Williamsburg, VA) following procedures described by Brasso and Cristol (Brasso and Cristol 2008) and Hawley et al. (Hawley et al. 2009). Briefly, total mercury (THg) was measured using cold vapor atomic absorption spectroscopy (Milestone DMA 80) and considered to be representative of methylmercury concentration because the ratio of THg to methylmercury in songbird blood and other tissues is nearly 1:1 (Gerrard and St. Louis 2001; Rimmer et al. 2005). Every 20 blood samples included two samples of standard reference materials (DORM-3 or DOLT-4), a method blank, a sample blank, and a sample replicate. Mean percent recoveries of the standard reference materials were 93.2 (1.4 %, DORM-3) and 97.1 (1.5 %, DOLT-4). Percent difference between the duplicate samples was <6 % (n=12). Interassay variation (CV) was 4.8 % (DORM-3) and 1.8 % (DOLT-4) for standard samples. Detection limit of the assay was 2.6 ppb. Results are reported in parts per million (milligrams per kilogram) wet weight.

Nestling sex determination

Sex was important to consider in our study because nestling growth in red-winged blackbirds is sex-dependent (Weatherhead et al. 2007). We molecularly sexed nestlings by extracting DNA from blood samples (DNeasy Tissue Kit, Qiagen, Valencia, California). We performed a polymerase chain reaction (PCR) with 1237 L (5′-GAGAAACTGTGCAAAACAG-3′) and 1296rev_Agpho (5′-CTTTCTGAGACKGAGTCA CTAT-3′) primers to amplify the Z and W sex chromosomes (Weatherhead et al. 2007). PCRs were carried out in 20-μL reaction volumes containing REDExtract-N-Amp™ master mix (Sigma-Aldrich, St. Louis, MO), 1 nM forward primers, 1 nM reverse primers, and 0.5–0.8 μg DNA. Details regarding the thermocycler conditions can be found in Fridolfsson and Fridolfsson and Ellegren (Fridolfsson and Ellegren 1999). We visualized PCR products in 4 % agarose gel containing 0.6 μg/mL ethidium bromide (Weatherhead et al. 2007). Samples from adults, whose sexes were known based upon plumage dimorphism, were used to validate the technique.

Statistical analyses

We tested for site differences in THg levels of adults and nestlings using two-tailed t tests and accepted significance at α=0.05. Growth rates for tarsus length, wing chord, and body mass were obtained from linear regressions of each morphometrics against nestling age at the time of measurement and then reduced into one variable (i.e., the first eigenvector; hereafter, "growth rate") with principal components analysis (PCA). We used linear mixed effects (LME) models with an information-theoretic approach to investigate the importance of THg, nestling sex, brood size, and brood identity for explaining variation in growth rate. Brood identity was considered as a random effect to account for the nonindependence of nestlings from the same brood, and mean brood size and nestling sex were examined as factors because of their potential to affect growth (Forbes and Glassey 2000; Weatherhead et al. 2007). Models built from all possible combinations of nestling THg, nestling sex, brood size, and brood identity were ranked according to Akaike's information criterion adjusted for small sample sizes (AIC_c). Akaike weights of all models in which a given variable appeared were then summed (Σw) and used to provide a relative measure of that variable's importance (Symonds and Moussalli 2011). We conducted the growth rate analyses with data from each study site individually and with data from the two sites pooled together to increase sample sizes. Results did not differ qualitatively between sites, and therefore, only results from the pooled analyses are presented. Linear mixed effect models were fitted to the data using SYSTAT 13. All other statistical analyses were performed in R (V.2.7.2). THg data were natural log-transformed prior to analyses to achieve normality; no other variables required transformation.

Results

We obtained blood samples and at least four successive morphological measurements from a total of 39 nestlings (21 male, 18 female) belonging to 16 different broods. Ten nestlings belonging to 4 broods were measured on the Bronx River, and 29 nestlings from 12 broods were measured at Kenridge Farm. Blood samples were taken from an additional 20 nestlings at Kenridge Farm, but not enough morphological measurements were obtained from these individuals to determine their growth rates. We also collected blood from five (three male, two female) adult red-winged blackbirds on the Bronx River and nine (four male, five female) adults at Kenridge Farm.

Adult red-winged blackbirds on the Bronx River had blood mercury levels between 0.036 and 0.117 ppm, and adults at Kenridge Farm had 0.055–0.746 ppm. Blood mercury levels of nestlings on the Bronx River ranged 0.012–0.115 ppm, while nestlings at Kenridge Farm had 0.009–0.284 ppm. Despite small sample sizes, there was a highly significant site difference in adult THg (Table 1), with birds at Kenridge Farm having nearly four times the blood THg level of those along the Bronx River. Nestling THg levels were also significantly higher at Kenridge Farm than along the Bronx River (Table 1).

The PCA of changes in nestling body mass, tarsus length, and wing chord produced three eigenvectors. The first eigenvector accounted for 67 % of the variation, representing overall nestling growth rate. All measurements had positive factor loadings (Table 2).

Among the LME model combinations, the global model was the highest ranked, and THg was also included in two of the three models with $\Delta AIC_c < 2$. However, the fit of each model in the candidate set was poor, including the top model which had a weight of only 0.31 (Table 3). The model containing only THg was one of the lowest ranked models and had no support (Table 3; Fig. 1). Summed weights indicated that sex contributed most ($\Sigma w = 1.00$) to the small amount of variation in growth rate that was explained by the models; male nestlings grew at 1.3 times the rate of females. THg had the lowest relative importance ($\Sigma w = 0.58$).

Discussion

Threshold mercury toxicity levels for passerine birds are poorly understood, and few studies have yet to directly investigate whether mercury accumulation in free-living passerines is great enough to cause adverse effects. Our study is one of only a few to investigate mercury levels of songbirds breeding in a major metropolitan area of North America and to relate songbird nestling mercury levels to their rate of growth—a key component of

Table 1 Site differences in total blood mercury concentrations (in parts per million) of adult and nestling red-winged blackbirds within (Bronx River) and outside (Kenridge Farm) New York City

	Bronx River	Kenridge Farm	t	d.f.	p
Adult	0.061±0.016 (n=5)	0.235±0.071 (n=9)	−3.029	12	0.010
Nestling	0.038±0.009 (n=10)	0.061±0.007 (n=49)	−2.070	57	0.043

Values are means±S.E.

Table 2 Scores of a principal components analysis of temporal changes in nestling red-winged blackbird body mass, tarsus length, and wing chord

	PC1	PC2	PC3
Body mass	0.601	−0.467	−0.649
Tarsus length	0.633	−0.218	0.743
Wing chord	0.488	0.857	−0.165
% variance	67	23	9

development and reproductive success. We found that blood mercury concentrations of adult red-winged blackbirds in the New York metropolitan area were well below those recently found to correspond with reduced reproductive success in another songbird, the Carolina wren (Jackson et al. 2011), and below or similar to those of conspecifics and other passerines in multiple nonurban regions of the northeastern USA (Evers et al. 2005; Evers et al. 2012; Rimmer et al. 2005; Edmonds et al. 2010; Osborne et al. 2011). We found no indication that blood mercury levels of nestlings affected their rate of growth.

Table 3 Rankings of linear mixed effect models for explaining variation in growth rates of nestling red-winged blackbirds. Models are arranged by Akaike's information criterion corrected for small sample sizes (AIC_c), with models with the highest AIC_c value considered to have the most support

Model	k^a	AIC_c	Δ_i	w_i
(1|Brood identity) + Mean brood size + Sex + THg	6	122.651	0.00	0.31
(1|Brood identity) + Mean brood size + Sex	5	123.115	0.46	0.25
(1|Brood identity) + Sex + THg	5	123.265	0.61	0.23
(1|Brood identity) + Sex	4	124.211	1.56	0.14
Sex + THg	4	128.68	6.03	0.02
Mean brood size + Sex	4	128.749	6.10	0.01
Mean brood size + Sex + THg	5	128.751	6.10	0.01
Sex	3	128.936	6.29	0.01
(1|Brood identity) + Mean brood size + THg	5	137.28	14.63	0.00
(1|Brood identity) + Mean brood size	4	137.58	14.93	0.00
(1|Brood identity) + THg	4	137.658	15.01	0.00
(1|Brood identity)	3	137.863	15.21	0.00
THg	3	140.198	17.55	0.00
Mean brood size+THg	4	140.212	17.56	0.00
Mean brood size	3	140.372	17.72	0.00

Brood identity was treated as a random effect and is indicated by parentheses. k^a is the number of fitted parameters in the model including the intercept and variance. Δ_i is the difference between the AIC_c of a given model and the highest ranked model. w_i is the Akaike weight and reflects the probability that a given model is the best approximating model (Symonds and Moussalli 2011)

Dietary intake of mercury by (or maternal transfer to) nestling red-winged blackbirds in our study sites may have been low, or sequestration of mercury in growing feathers may have protected nestlings from reaching harmful blood mercury concentrations. Longcore et al. (2007) and Gerrard and St. Louis (2009) also found that the growth rates of nestling passerines (tree swallows; *Tachycineta bicolor*) were unaffected by mercury. Mercury easily binds to keratin, and feathers provide a biologically inert site into which ingested mercury can be shunted (Condon and Cristol 2009). Sequestration of mercury in feathers can prevent toxic levels of mercury from accumulating in living tissues, thereby protecting young birds from mercury early in their development. Once feather growth is complete, however, mercury levels in blood and other tissues may rise and begin to impair development (Spalding et al. 2000; Kenow et al. 2003; Condon and Cristol 2009; Ackerman et al. 2011). We cannot determine from our study whether mercury negatively affected the growth of red-winged blackbirds beyond the age range we monitored.

Average blood mercury levels of nestlings in our study sites were higher than those of red-winged blackbird nestlings in the nearby New Jersey Meadowlands (Tsipoura et al. 2008), a large wetland complex that has been contaminated and degraded by surrounding industrial land use, possibly indicating that exposure of red-

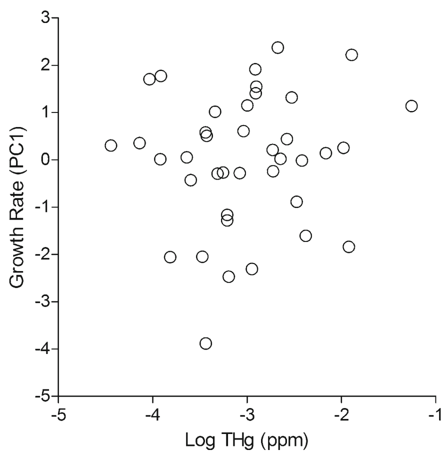

Fig. 1 Linear model of growth rate (*PC1*) and blood mercury level (*THg*) in nestling red-winged blackbirds in Cornwall and Bronx, New York, 2010 (R^2=0.01, N=39, P=0.52)

winged blackbirds to mercury in this and other wetlands throughout the New York Harbor region is also not enough to impair nestling development. The blood THg levels of the adults in our study were well below those of red-winged blackbirds nesting in wetland habitats elsewhere in the northeastern USA and in maritime Canada (Evers et al. 2005; Edmonds et al. 2010), and instead, were more comparable to various insectivorous passerine species breeding in high-elevation montane forests of New England (Rimmer et al. 2005).

Interestingly, mercury concentrations of red-winged blackbirds in our study site outside of the city exceeded those of birds along the lower Bronx River by nearly fourfold. Although atmospheric mercury deposition is widespread throughout the northeastern USA (Dennis et al. 2005; Driscoll et al. 2007), we expected birds along the Bronx River to have greater blood mercury concentrations than birds at Kenridge Farm because of the river's urban surroundings and history of industrial uses. However, rivers tend to retain mercury less than other aquatic systems due to their ability to continually flush contaminants (Evers et al. 2005), and it is possible the hydrological regime of the Bronx River prevents any additional local mercury inputs from accumulating. In contrast, the small ponds of Kenridge Farm may act as terminal basins for mercury accumulation and methylation. We observed adult red-winged blackbirds consuming and feeding predatory invertebrates, such as dragonflies, to nestlings at Kenridge Farm more often than at the Bronx River site. Predatory invertebrates are more likely to biomagnify mercury (Cristol et al. 2008) and this may also partially account for the higher THg concentrations of birds at Kenridge Farm.

Although our results suggest that mercury accumulation by the nestlings we studied was below the concentration required to have deleterious effects on their growth, more work needs to be done before songbird mercury levels observed in the wild can be accurately and confidently interpreted. Laboratory dosing studies will substantially improve our understanding of how mercury is impacting wild songbirds. Lowest observable effect levels will need to be established for multiple species because considerable interspecific variation in mercury sensitivity is likely (Heinz et al. 2009). Intraspecific differences in mercury sensitivity, among populations or among families within a population, are also possible (Varian-Ramos et al. 2013). For these reasons, we caution that although the growth of red-winged blackbird nestlings in our study sites did not appear to be affected by mercury, it remains possible that other passerines breeding in these areas are accumulating harmful levels of mercury and that nestling red-winged blackbirds in other populations are differently affected by similar levels of mercury exposure. In addition, it should be noted that mercury levels of the nestlings we studied may have been insufficient to affect their growth rate but great enough to cause neurological damage or other unseen adverse effects.

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