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NOTE

Blood mercury levels and the stopover refueling performance of a long-distance migratory songbird

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Abstract: I examined the relationship between total mercury (THg) and plasma triglyceride (TRIG; an indicator of body mass change) levels in the blood of migrating Northern Waterthrushes (*Parkesia noveboracensis* (Gmelin, 1789)) to test the hypothesis that mercury has a negative influence on the stopover refueling rates of migratory birds. THg levels averaged 0.42 ppm and ranged 0.09–2.08 ppm. Model selection indicated that THg was not important for explaining variation in TRIG relative to capture time, body mass, and year. Summed model weights also indicated that THg had low relative importance. Capture time appeared alone in the global best model and had the greatest relative importance. Subsets of birds in the 25th and 75th percentiles of THg level did not have different levels of TRIG. THg in most birds was higher than mean blood levels reported for several other long-distance migrants from the same geographic region, but below the lowest blood level recently determined to cause adverse effects (reduced reproductive success) in a passerine (0.7 ppm). Blood THg levels in this study did not seem to affect foraging efficiency or other attributes of Northern Waterthrushes enough to reduce their stopover refueling rate. Research is needed to identify mercury effect levels for neurological, physiological, and behavioral changes that would impair the migration performance of passerine birds.

Key words: mercury, songbird, migration, stopover refueling, triglyceride, *Parkesia noveboracensis*, Northern Waterthrush.

Résumé : J'ai examiné le lien entre le mercure total (THg) et les concentrations de triglycérides plasmatiques (TRIG, un indicateur de changement de la masse corporelle) dans le sang de parulines des ruisseaux (*Parkesia noveboracensis* (Gmelin, 1789)) en migration afin de tester l'hypothèse selon laquelle le mercure a une incidence négative sur les taux de ravitaillement durant les haltes des oiseaux migrateurs. Les valeurs de THg allaient de 0,09 à 2,08 ppm pour une moyenne de 0,42 ppm. La sélection de modèles indiquait que le THg n'était pas un facteur explicatif important des variations des concentrations de TRIG en fonction du moment de la capture, de la masse corporelle ou de l'année. La somme des poids des modèles indiquait également une faible importance relative du THg. Le moment de la capture semblait être la seule variable explicative dans le meilleur modèle global et avait la plus grande importance relative. Les concentrations de TRIG des sous-ensembles d'oiseaux des 25e et 75e centiles de la concentration de THg n'étaient pas différentes. La teneur en THg dans la plupart des oiseaux était supérieures aux teneurs signalées pour plusieurs autres espèces de grands migrateurs de la même région géographique, mais plus faibles que la teneur sanguine minimum pouvant causer des effets néfastes (diminution du succès de reproduction) chez des passereaux récemment établie à 0,7 ppm. Les concentrations sanguines de THg observées dans le cadre de l'étude ne semblaient pas avoir une incidence suffisante sur l'efficacité de la quête de nourriture ou d'autres attributs des parulines des ruisseaux pour causer une diminution du taux de ravitaillement durant les haltes migratoires. Des travaux sont nécessaires pour déterminer les concentrations de mercure entraînant des changements neurologiques, physiologiques et comportementaux qui réduiraient la performance migratoire des passereaux. [Traduit par la Rédaction]

Mots-clés : mercure, oiseau chanteur, migration, ravitaillement durant les haltes, triglycéride, *Parkesia noveboracensis*, paruline des ruisseaux.

Introduction

There is growing concern that passerine birds (songbirds) that primarily feed on insects, arachnids, and other macroinvertebrates are accumulating harmful levels of mercury (Seewagen 2010; Evers et al. 2012). By comparing birds at sites with heavy point-source mercury contamination to those at reference sites, recent work has demonstrated that mercury uptake by free-living, terrestrial passerines can be enough to adversely affect endocrine function (Wada et al. 2009), immunocompetence (Hawley et al. 2009), song quality (Hallinger et al. 2010), and reproductive success (Brasso and Cristol 2008), and skew sex ratios of offspring (Bouland et al. 2012). From a study of Carolina Wrens (*Thryothorus ludovicianus* (Latham, 1790)), Jackson et al. (2011a) recently provided a first indication of the threshold mercury level that is necessary to impair reproductive success in passerines, but effect levels have yet to be well established for other passerine species or for other aspects of a passerine bird's health, condition, or fitness.

One area that remains unstudied is how mercury burdens of passerines, many of which are long-distance migrants, affect their migration performance or success. Several consequences of sublethal mercury toxicity that have been documented in large-bodied, nonpasserine birds, such as flight feather asymmetry, reduced oxygen carrying capacity of the blood, ataxia, appetite suppression, and reduced motivation to forage, give reason to expect that mercury could have negative effects on migration

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during flight and (or) stopover phases (Seewagen 2010). A primary determinant of migration success for passerines is their ability to promptly and efficiently refuel during stopovers (Alerstam and Hedenström 1998); yet it remains unknown whether the mercury levels observed in wild passerines are capable of influencing their stopover refueling performance (Seewagen 2010).

During the autumn of 2008 and 2009, I measured blood plasma triglyceride levels (an indicator of refueling rate) of Northern Waterthrushes (*Parkesia noveboracensis* (Gmelin, 1789)) and other migrating songbirds in the New York metropolitan area as part of an effort to assess the quality of urban parks as stopover habitats (Seewagen et al. 2011). I concurrently collected separate blood samples from many of the same Northern Waterthrushes to contribute to a mercury monitoring network aimed at improving our understanding of the extent and spatial distribution of mercury accumulation in North America's songbirds (see Evers et al. 2012). This afforded an additional opportunity to examine the relationship between these variables. Here, I use the intraindividual measures of blood mercury and plasma triglyceride levels in the Northern Waterthrush to investigate the influence of mercury on the instantaneous stopover refueling rates of a migratory bird. I predicted that birds with low blood mercury levels would exhibit faster rates of stopover refueling relative to birds with higher blood mercury levels.

Materials and methods

Study species and field methods

The Northern Waterthrush is a Neotropical migratory passerine that winters in Latin America and the Caribbean, and breeds throughout the northern US and Canada. It primarily feeds on benthic invertebrates probed from stream edges and floodplains, where methylation of elemental mercury can be high (Evers et al. 2005). Blood samples were collected from 30 Northern Waterthrushes during autumn stopovers in Bronx Park and Prospect Park, New York City, which contain deciduous woodlands that are well known for receiving large concentrations of migrating songbirds and are of the same quality as stopover habitat (Seewagen et al. 2011). The birds used in this study were captured in mist nets between 0700 and 1145, from 8 September to 2 October 2008 and 2009 (14 birds were sampled in 2008 and 16 birds were sampled in 2009). Nets were checked every 8 min or less so blood could be collected before plasma triglyceride level (hereafter TRIG) responded significantly to a change in feeding state (Guglielmo et al. 2005; Zajac et al. 2006). Birds were assumed to have been in the net the entire time since the net was last checked and "bleed time" was conservatively measured as the time elapsed between the previous net check and blood-sample collection. All bleed times were under 20 min (11 ± 5 min, mean ± SD) and TRIG levels were therefore considered to be reflective of the birds' foraging activity prior to capture (Guglielmo et al. 2005; Zajac et al. 2006).

Up to 100 μL of blood was collected by brachial veinipuncture with a 26-gauge needle into heparinized capillary tubes and stored in a cooler for the remainder of the morning. Samples for TRIG analysis were then centrifuged for 5 min and plasma was transferred to 0.6 mL cryogenic tubes. Plasma was stored at −80 °C for up to 3 months until analysis. Samples for mercury analysis were stored as whole blood at −80 °C until the conclusion of the field season and then shipped to an analytical laboratory.

After blood sampling, birds were banded with a US Geological Survey aluminum leg band, assigned to an age class when possible (Pyle 1997), measured (unflattened wing length to 1 mm), weighed to 0.1 g on a digital balance, and then released. Field methods were approved by the University of Western Ontario's Animal Use Subcommittee and the Wildlife Conservation Society's Institutional Animal Care and Use Committee.

Indicators of refueling rate and mercury accumulation

TRIG concentrations were used to measure Northern Waterthrush rates of stopover refueling. TRIG rises rapidly in birds when dietary or de novo synthesized lipids are shuttled through the bloodstream to storage sites (Jenni-Eiermann and Jenni 1998). TRIG responds to subtle increases in body mass (Jenni-Eiermann and Jenni 1994; Zajac et al. 2006) and thus provides a robust indicator of stopover refueling rate in field studies of birds (e.g., Guglielmo et al. 2005; Anteau and Afton 2008).

Blood mercury level was used as an indicator of overall body burden. Blood mercury level is usually measured to reflect recent and local exposure (Evers et al. 2005), but during migration, blood mercury may be partly indicative of longer-term exposure (i.e., on wintering and breeding or natal grounds) because lean tissues that accumulate mercury over time (Spalding et al. 2000a; Kenow et al. 2007) are catabolized in flight (Bauchinger and Biebach 1998; Gerson and Guglielmo 2011), even when birds do not have to cross geographical barriers or otherwise fly nonstop for excessive amounts of time (Gerson and Guglielmo 2011; Seewagen and Guglielmo 2011; Klaassen et al. 2000). Mercury accumulated elsewhere and bound to these tissues is likely released into the bloodstream, resulting in circulating mercury beyond that which is solely attributable to recent exposure at a given stopover site (Seewagen 2010; see also Klaassen et al. 2012). This is analogous to the mobilization of lipophilic toxicants during in-flight lypolysis (Klaassen et al. 2012; Scollon et al. 2012) and the mobilization of mercury from muscle when muscle tissue is used to fuel feather growth (Evers et al. 2005).

Laboratory analyses

Samples for mercury analyses were sent to the Biodiversity Research Institute (19 Flaggy Meadow Road, Gorham, ME 04038, USA) where total mercury (THg) was measured (based on wet weight) using thermal decomposition with an automated direct analyzer (DMA 80, Milestone Inc., Shelton, Connecticut, USA; US EPA Method 7473). The laboratory's protocol includes tests of two standard reference materials (Dorm-3 and Dolt-4), two method blanks, and one sample blank before and after each set of 20 samples is analyzed. One sample out of 20 is tested in duplicate to confirm a relative percent difference between replicates of <10% (for additional details see Jackson et al. 2011a, 2011b). TRIG was analyzed by the author and measured as the difference of free and triacylglycerol-bound glycerol concentrations, as determined by endpoint assay (Sigma Trinder reagent A and B) on a microplate spectrophotometer (Powerwave X340, Biotek Instruments Inc., Winooski, Vermont, USA) (Guglielmo et al. 2005; Seewagen et al. 2011). Samples were analyzed in duplicate and averaged (all coefficients of variation were <15%).

Statistical analyses

I used general linear models with an information–theoretic approach to evaluate the importance of THg, year, time of day (hours since sunrise at the time of capture), and body mass for explaining variation in TRIG. To avoid the shortcomings and explorative nature of a large variable set when using information–theoretic methods (Burnham and Anderson 2002; Symonds and Moussalli 2011), I excluded Julian date of capture, study site, and age, which previous work at these sites indicated were generally not important factors contributing to TRIG levels of autumn migrants (Seewagen et al. 2011, 2013). Exploratory analyses also indicated that TRIG levels of the Northern Waterthrushes used in this study were not related to Julian date (Spearman's rank correlation: r = 0.08, P = 0.67), study site (Student's t test: $t_{[28]}$ = 0.95, P = 0.35), or age (Student's t test: $t_{[19]}$ = 1.73, P = 0.10). I excluded Julian date and age also because of the narrow range within the migration period during which samples were collected and because some birds could not be confidently assigned to an age class. In the Northern

Table 1. Rankings of models for explaining variation in refueling rates (plasma triglyceride levels) of Northern Waterthrushes (*Parkesia noveboracensis*) during stopovers in New York City, USA, during autumn 2008 and 2009 (n = 30) based on Akaike's information criterion corrected for small sample size (AIC_c).

Model	k^a	AIC_c	Δ_i	w_i
Time	3	4.40	0.00	0.24
Time + body mass	4	4.78	0.37	0.20
Year + time	4	5.22	0.81	0.16
Body mass	3	5.91	1.50	0.11
Year + time + body mass	5	7.03	2.62	0.06
Time + THg	4	7.53	3.12	0.05
Year	3	7.68	3.27	0.05
Year + body mass	4	8.40	3.99	0.03
Time + THg + body mass	5	8.40	4.00	0.03
Year + time + THg	5	9.12	4.72	0.02
THg + body mass	4	9.63	5.22	0.02
THg	3	9.70	5.30	0.02
Year + time + THg + body mass	6	11.15	6.75	0.01
Year + THg	4	11.23	6.83	0.01
Year + THg + body mass	5	12.32	7.91	0.00

Note: Time represents hours after sunrise at time of capture; THg represents total blood mercury level.

[a]Number of parameters in the model, including variance and intercept.

Waterthrush, adults molt and juveniles complete feather growth on breeding grounds at similar times, before the onset of autumn migration (Eaton 1995; Pyle 1997). Thus, no substantial age differences in the ability to depurate mercury into growing feathers should have recently existed among the birds studied. Of the subset of Northern Waterthrushes that were aged, exploratory analyses found that hatch-year and after-hatch-year birds did not have significantly different blood THg levels (Student's t test: $t_{[19]} = 1.26$, $P = 0.22$).

Models built from all possible combinations of THg, year, time, and body mass were ranked according to Akaike's information criterion corrected for small samples sizes (AIC_c). Akaike weights summed across all models in which a given parameter appeared were then used to provide a relative measure of that parameter's importance (Symonds and Moussalli 2011); directions of effects were determined with simple linear regressions. I also explicitly compared TRIG between groups of birds in the 25th (<0.20 ppm) and 75th (>0.44 ppm) percentiles of THg level using analysis of covariance (ANCOVA), with variables with the greatest relative importance values included as covariates.

Body mass was not adjusted to body size (wing length) because they were unrelated (simple linear regression: $r^2_{[1,28]} = 0.00$, $P = 0.80$). THg and TRIG data were transformed by $\log_{10}(x + 1)$ to achieve normality. All analyses were conducted using SYSTAT version 12 (Systat Software Inc., Chicago, Illnois, USA). Values are reported as means ± SE.

Results

Blood THg averaged 0.42 ± 0.08 ppm and ranged 0.09–2.08 ppm. TRIG averaged 1.46 ± 0.18 mmol·L^{-1} and ranged 0.41–4.80 mmol·L^{-1}. Time of day was the only variable that appeared in the model that best described variation in TRIG. Three other models were well supported ($\Delta AIC_c < 2$) and contained time of day, body mass, and (or) year. All models containing THg had low support (Table 1). Summed weights indicated that THg (0.15) and year (0.33) were not important factors for explaining variation in TRIG, whereas time of day (0.76) and body mass (0.46) had high and moderate levels of relative importance, respectively. TRIG increased with time of day and body mass (Fig. 1), and had no relationship with THg (Fig. 2). TRIG of birds in the 25th (n = 8) and 75th (n = 8) percentiles of THg level were not different, after controlling for time of day ($F_{[2,13]} = 1.32$, $P = 0.72$).

Fig. 1. Regressions of blood plasma triglyceride level (TRIG; an indicator of refueling rate) on body mass (A) and time of capture (B) in Northern Waterthrushes (*Parkesia noveboracensis*) captured during stopovers in New York City, USA, during autumn 2008 and 2009 (n = 30).

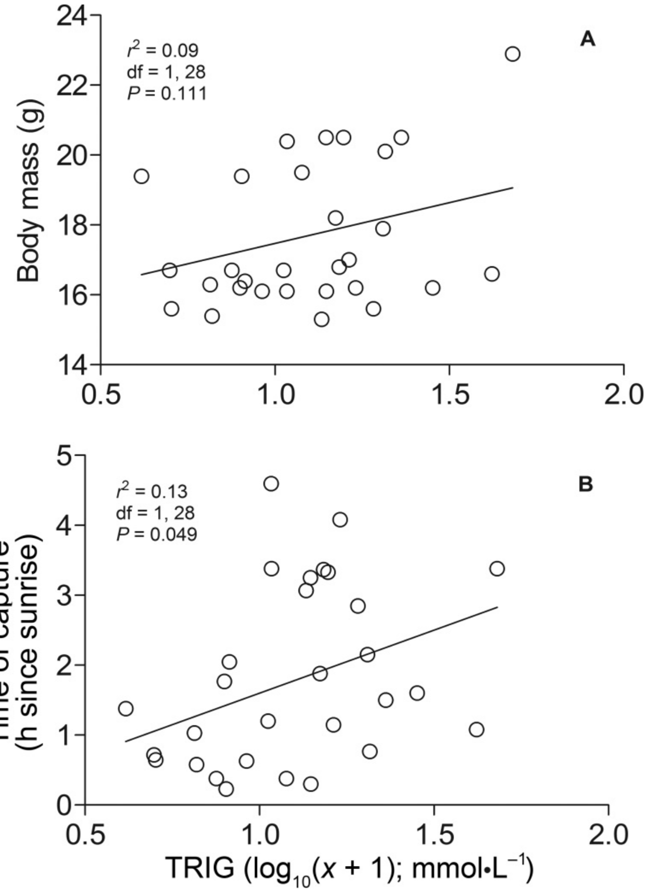
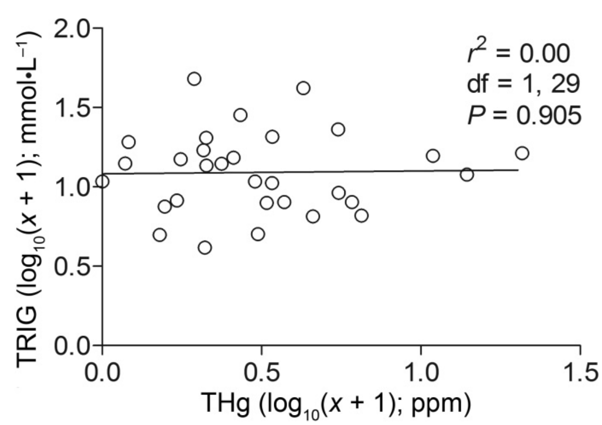


Fig. 2. Regression of total blood mercury level (THg) on blood plasma triglyceride level (TRIG; an indicator of refueling rate) in Northern Waterthrushes (*Parkesia noveboracensis*) captured during stopovers in New York City, USA, during autumn 2008 and 2009 (n = 30).



Discussion

Northern Waterthrushes showed no indication that their stopover refueling rate was affected by circulating THg levels. Variation in TRIG, a strong indicator of refueling rate, was not explained by THg and did not differ between subsets of birds with the highest and lowest THg levels of those examined. Time of capture had the greatest influence on TRIG, likely representing the transition from overnight mass loss to morning refueling (Guglielmo et al. 2005; Seewagen et al. 2011). To my knowledge, the influence of mercury on the stopover refueling rates of migratory birds has not been previously investigated. Mercury levels in the livers of migrating ducks and loons were found to be unrelated and negatively related, respectively, to their fat mass (Anteau et al. 2007; Schummer et al. 2012), but static measures of body composition do not reflect the time it took a bird to acquire its current energetic condition and therefore do not provide a true measure of refueling performance (Williams et al. 1999; Anteau and Afton 2008). Additionally, the migration of waterbirds, which stage for long periods of time, and that of songbirds, which make frequent and short stopovers where refueling must occur rapidly, are highly disparate and may not be affected by mercury in similar ways or to similar extents.

Most birds had blood THg levels that were above the mean levels reported for Northern Waterthrushes and numerous other long-distance migratory passerines elsewhere in the eastern US (Osborne et al. 2011), but well below the level that corresponded with a 10% reduction in the reproductive success of Carolina Wrens and is currently used as a general marker of risk in passerines (0.7 ppm; Jackson et al. 2011a; Evers et al. 2012). The few other studies that have documented adverse effects of mercury in passerines have done so by comparing birds in highly contaminated sites to conspecifics in reference areas, which does not provide a threshold effect level for the study species. For example, Tree Swallows (*Tachycineta bicolor* (Vieillot, 1808)) with mean blood THg levels of >2.5 ppm in mercury-polluted sites had suppressed immune competence relative to Tree Swallows with mean blood THg levels of 0.16 ppm in reference sites (Hawley et al. 2009), but it could not be determined at what mercury level such effects began to occur. Similarly, at contaminated sites where blood mercury levels of Tree Swallow averaged 3.56 ppm, females breeding for the first time had lower reproductive success than those in reference sites where blood mercury levels of Tree Swallow averaged only 0.17 ppm (Brasso and Cristol 2008), but a threshold level for reproductive impairment could not be established. Unfortunately, studies that have determined effect levels for birds (nonpasserines) have rarely reported blood mercury values, and have instead reported mercury levels in organs and other tissues measured after study subjects died (Seewagen 2010). Effect levels for blood mercury that have been determined in young birds with actively growing feathers (e.g., Spalding et al. 2000b) have limited relevance to fully developed juvenile and nonmolting adult birds because of the protection from mercury toxicity provided by feather growth (Condon and Cristol 2009). For these reasons, the implications of blood mercury levels observed in wild songbirds remain difficult to interpret.

It is further unknown what blood mercury concentration in passerines is needed to cause the particular kinds of neurological, physiological, or behavioral changes that would reduce stopover refueling performance. Great Egrets (*Ardea alba* L., 1758) dosed to mean blood THg levels of 11.9 and 12.3 ppm had reduced appetite and motivation to forage relative to control subjects, but an unimpaired ability to capture prey (Bouten et al. 1999; Spalding et al. 2000a). From this study, it seems that blood THg levels ranging from 0.09 to 2.08 ppm did not affect motivation to forage, foraging efficiency, or other physical or behavioral attributes of Northern Waterthrushes enough to limit their stopover refueling rate. Interestingly, the individual with the highest and well above the mean blood THg level of 2.08 ppm had a TRIG level (1.63 $mmol \cdot L^{-1}$) that was also above the mean value for the study group and comparable with, or above, the mean TRIG levels of several other species studied at these sites during autumn migration (Seewagen et al. 2011).

These findings from a modest sample size of one species need to be taken with caution, and generalization to other species is not recommended because of likely interspecific differences in mercury sensitivity (Heinz et al. 2009). The potential for mercury to impact stopover refueling could also be dependent upon habitat quality; effects may not have been detected in this study if the sites examined had plentiful food resources and foraging was not demanding. In addition, mercury may affect other aspects of migration beyond stopover refueling, such as flight efficiency and navigation. Further research is certainly needed to clearly assess the influence mercury may have on the migration performance of birds. Sublethal dose–response laboratory studies would promise to substantially improve interpretation of the significance of mercury levels found in free-living passerines during migration or any other phase of their life cycle. Wind tunnel or at-rest fasting studies that document to what extent protein-bound mercury is released into the bloodstream during lean tissue catabolism would also be beneficial towards understanding the threats of mercury to migrating birds.

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