Mercury and nestling growth rates of red-winged blackbirds (*Agelaius phoeniceus*) in the New York metropolitan area

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ABSTRACT

Mercury has been primarily studied in piscivorous birds, but recent evidence shows that its bioaccumulation and biomagnification in invertebrate prey is placing insectivorous passerines at risk for heightened exposure. The effects of mercury remain poorly understood in these species. As nestling growth is crucial to the survival and fitness of individuals after fledging, this study seeks to understand the impact of mercury on nestling growth rates in the red-winged blackbird (*Agelaius phoeniceus*). I chose to study this topic within the red-winged blackbird because research shows evidence of the species' susceptibility to mercury accumulation at concentrations higher than those found in some fish-eating birds. I captured, monitored, and blood sampled individuals from two populations breeding in the New York metropolitan area to determine levels of mercury exposure. Nestlings were measured every other day to calculate linear growth rates of mass, tarsus length, and wing chord to in turn, determine whole body growth. I found no relationship between mercury and whole body growth. Although I did not measure mercury in feathers, I surmise from previous studies that the sequestration of mercury in growing feathers protected the nestling from toxicity. However, the effects of elevated mercury exposure after fledging warrants further research.

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INTRODUCTION

Recent evidence indicates that invertebrate prey items are bioaccumulating and biomagnifying mercury and in turn, subjecting insectivorous passerines to high levels of exposure (Cristol et al. 2008). A growing body of literature demonstrates that mercury concentrations in insectivorous passerines associated with freshwater systems are similar to or higher than those found in piscivorous avifauna (Brasso & Cristol 2008; Evers et al. 2005; Tsipoura et al. 2008; Gerard & St. Louis 2001; Longcore, Haines & Halteman 2007b; Cristol et al. 2008; Seewagen 2009). For example, insectivorous songbirds like the marsh wren (*Cistothrous palustris*) and tree swallow (*Tachycineta bicolor*) have exhibited elevated mercury concentrations in feather and blood samples comparable to those of larger bodied species occupying higher trophic positions (Brasso & Cristol 2008; Tsipoura et al. 2008). At a superfund site, mercury in tree swallow eggs reached concentrations that exceeded embryotoxic levels (Longcore et al. 2007b).

Although these studies have focused on mercury in wetland ecosystems, bioavailable methylmercury (MeHg) is also prevalent in terrestrial habitats (Cristol et al. 2008; Rimmer et al. 2010). Cristol et al. (2008) observed mercury levels in 13 terrestrial-feeding species that rivaled those in five aquatic-feeding species. Of these, the species with the highest concentrations were two terrestrial songbirds, the red-eyed vireo (*Vireo olivaceus*) and Carolina wren (*Thryothorus ludovicianus*) (Cristol et al. 2008). MeHg has also been detected in blood samples of forest birds, including a montane forest specialist the Bicknell's thrush (*Catharus bicknelli*) (Rimmer et al. 2005). While it is becoming increasingly evident that mercury is widespread in insectivorous passerines at levels that may be harmful to their survival and reproduction, it remains unknown what

concentrations of mercury cause the sublethal adverse effects seen in other well researched species (Seewagen 2009).

Recent studies have related mercury to reductions in immunocompetence (Hawley et al. 2009), hatching and fledging success (Brasso & Cristol 2008), thyroid and stress hormones (Wada et al. 2009), and diversity in song production (Hallinger et al. 2010). Among these, one study found no adverse effects of mercury exposure on nestling growth (Longcore et al. 2007a). However, negative effects have been observed in other avian species while under dosing regimens and laboratory conditions (Fimreite & Karstad 1971; Spalding et al. 2000). As normal growth rates and fledging weight is crucial to nestling survival and fitness post-fledging (Gebhardt-Henrich & Richner 1998; Greno et al. 2008), I believe this topic warranted further attention.

I examined the relationship between mercury exposure and nestling growth in the red-winged blackbird (*Agelaius phoeniceus*), a common songbird that nests in wetland habitats. Although a large body of knowledge exists on its ecology and life history, little is known about the adverse effects of mercury in this species as most studies simply report levels of exposure. For example, previous research shows evidence of this species' susceptibility to mercury accumulation at concentrations higher than those found in some fish-eating birds (Evers et al. 2005; Seewagen 2009; Tsipoura et al. 2008). I expected this as adults primarily feed on invertebrates during breeding, but they may also consume grain and other types of animal matter (Yasukawa & Searcy 1995). Nestlings are similarly, if not more, vulnerable to exposure for their diets consist solely of emerging aquatic insects and terrestrial arthropods (e.g., odonates and dipterans; Yasukawa & Searcy 1995), which are known to accumulate mercury (Brasso & Cristol 2008).

I monitored nests in an urban and suburban environment in the New York metropolitan area because there is evidence of heightened atmospheric mercury deposition in the northeastern United States (Driscoll et al. 2009). I expected greater mercury burdens at the urban site, as higher levels of atmospheric mercury is known to occur in this environment (Landis, Vette & Keeler 2002; Dennis et al. 2005). Levels of exposure were expected to be elevated to concentrations that would cause adverse effects on nestling growth. Based on evidence from previous studies (Fimreite & Karstad 1971; Spalding et al. 2000), I hypothesize that individuals with high mercury loads will exhibit reduced growth rates relative to individuals with lower loads.

METHODS AND MATERIALS

Study Site Descriptions:

I collected data from red-winged blackbirds nesting along the Bronx River, New York, (40º51′N, 73º52′W) and at Kenridge Farm, Cornwall, New York (41º25′N, 74º02′W) to examine levels of mercury exposure and nestling growth rates among a population of an urban site and that of a suburban site.

Field site 1: Bronx River, New York

The Bronx River begins at the Kensico Dam in Valhalla, NY, spans 23 miles south, and empties into the East River, NY (Samaritan & Schmidt 1982). It is the only freshwater river in New York City. However, the quality of its water remains in question, as it has endured a long history of contamination from urban and industrial sources (Zimmerman 2002). In the 1840s, construction of the New York Central Railroad led the

river to be utilized as an industrial corridor for the transportation of coal-powered trains (Zimmerman 2002). Since mercury is emitted from coal-burning (Evers et al. 2007), this could have been a likely source of past mercury deposition along the river. Effluents from combined sewers, which collect industrial wastewater as well as domestic sewage, have also polluted the river (Zimmerman 2002).

Fieldwork was conducted from May 13 to July 3, 2010 every other day (weather permitting) along a section of the river that bordered the west side of the Bronx Zoo. In order to re-locate nests, I set up randomly dispersed plot markers to act as standard points from which directions to nests can be written. I searched for nests in the river's riparian zone, in which willow (*Salix* spp.), swamp dogwood (*Cornus foemina*), and stinging nettles (*Urtica dioica*) dominated, as well as two non-native species, i.e. Japanese knotweed (*Polygonum cuspidatum*) and Oriental bittersweet (*Celastrus orbiculatus*) (Seewagen & Slayton 2008). This habitat is flanked by dry upland deciduous forest of mostly red oak (*Quercus rubra*) and sweet gum (*Liquidambar styraciflua*) (Seewagen & Slayton 2008).

Field site 2: Kenridge Farm, Cornwall, New York, USA

Kenridge Farm is located approximately fifty miles northwest of the Bronx River within the Hudson Highlands. The 177-acre property began as pastureland in the 1700s, was then converted to farmland in the early 1800s, and currently remains as managed farmland under the trust of the Hudson Highlands Nature Museum (pers. com. Bill Schuster). The farm is surrounded by suburban development. It harbors many native and

non-native grasses like cattail (*Typha spp.*), smooth bromegrass (*Bromus inermis)*, and common reed (*Phragmites australis*).

Fieldwork occurred from May 29 to July 29, 2010 every other day with weather permitting. If bad weather occurred, I delayed fieldwork on that site until the following day. Nests were found along the vegetated edges or in the floodplains surrounding multiple managed ponds.

The wetland system of Kenridge Farm is composed of a mix of permanent and ephemeral ponds that sustain themselves through snowmelt, rainfall, and groundwater inputs. Although the highly polluted Hudson River is nearby (Feng et al. 1998), it shares little to no hydrologic connectivity with the ponds as they are located 3.6 km southwest of the river at an elevation of 94m. The ponds may be affected by the river through atmospheric inputs of contaminants (Poissant et al. 2000), but this is most likely negligible compared to the direct mercury discharges that riparian systems receive (e.g. the Bronx River field site).

Field Data Collection

Nest monitoring

I searched for and monitored nests every other day at each field site in compliance with the BBIRD protocol (Martin et al. 1997). Nests were numbered and mapped; pink flagging was placed ~5m away from the nest to assist in relocation.

I measured 39 nestlings from 15 broods between ages 0 and 11 days for body mass, tarsus length, and wing chord every other day (see Longcore et al. 2007a for descriptions of measurements). Nestlings were measured at least 4 times to determine

growth rates. All morphological measurements were taken with Ultra-Cal Mark IV electronic calipers. Before nestlings could be banded, I marked individuals on both tarsi with black, green, or blue non-toxic marker to facilitate identification. Red or orange markings were avoided to prevent anomalous parental behaviors because shades of red have been shown to influence male territorial aggression (Metz & Weatherhead 1991). When nestlings reached 8 or 9 days old, I banded each with a United States Fish and Wildlife Service (USFWS) aluminum band. When nests were found with chicks, I estimated ages based on physical features like feather pin size and degree of eye opening to determine when nestlings could be banded (Holcomb & Twiest 1971; Tsipoura et al. 2008).

Mist netting

I captured 14 red-winged blackbird adults using mesh nylon mist nets throughout the season. To attract individuals into nets, I elicited males using playbacks of vocalizations and caught females who were incubating or feeding nestlings.

Blood sampling

I collected blood samples from 14 adults and 59 nestlings belonging to 22 broods in order to assess the most recent levels of mercury exposure to local food sources (Evers et al. 2005). I took samples from 8 or 9 day old nestlings with 26 gauge needles (Becton Dickinson and Company, Franklin Lakes, NJ) from the medial metatarsal vein or cutaneous ulnar vein, while those from adults came only from the cutaneous ulnar vein. No more than 1% of the body weight of the animal was collected at any one time (Gaunt

& Oring 1999). I transported samples in a cooler with ice packs and froze them on the same day. Samples were stored at -20ºC until analyzed.

Mercury Analysis

Blood samples were analyzed at the College of William and Mary, VA. A Milestone® DMA 80 using cold vapor atomic absorption spectroscopy was used to analyze samples for total mercury concentrations (Brasso & Cristol 2008). Total mercury (THg) then reflects MeHg concentrations because MeHg composes 95% of THg in songbird blood (Rimmer et al. 2005). Every 20 samples included two samples of each standard reference material (DORM-3 or DOLT-4), a method blank, a sample blank, and a sample replicate. Sample replicates "were obtained by comparing two capillary tubes of blood from the same collection of the same bird run in the same batch" (Hawley et al., 2009). 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 less than 6% (n=12). Inter-assay variation (i.e. % coefficient of variation) 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 (mg/kg) wet weight.

Nestling Sex Determination

Sex differences in growth are evident in red-winged blackbirds (Weatherhead et al. 2007). To control for this, I determined the sex of each nestling using molecular genetics techniques. With the DNeasy Tissue Kit (Qaigen, Valencia, California), I extracted DNA from the residual blood samples left after mercury analysis and then performed the

polymerase chain reaction (PCR) with 1237L (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), 1nM forward primers, 1nM reverse primers, and 0.5-0.8μg DNA. Details regarding the thermocycler conditions can be found in Fridolfsson and Ellegren (1999). I 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, were used as controls to ensure the validity of results.

Statistical Analysis

Data analyses were performed in R (V.2.7.2). I used ANOVA to determine if blood THg concentrations differed between locations within adults and then within nestlings. Because no difference in adult and nestling THg concentrations were found between populations at Kenridge Farm and the Bronx River, I pooled all individuals into one data set and used ANOVA to examine how concentrations differ between adults and nestlings and differ between sexes.

I then calculated the linear growth rates of mass, tarsus length, and wing chord from all measured nestlings. I used the rate of change (i.e. slope of the linear model) of each morphological feature as an estimate of growth.

To analyze the relationship between mercury exposure and nestling growth, I related blood THg concentrations to whole body growth rather than to each measurement individually. I performed a principal components analysis (PCA) to decompose these

three measurements into a single response variable, i.e. the first eigenvector, named herein as "body growth." The effect of mercury exposure on nestling body growth was then assessed using a linear mixed effects (LME) model with residual maximum likelihood estimation (nlme package, lme-function). To account for the non-independence of nestlings from the same brood, I denoted the identity of the brood as the random effect. Mean brood size (i.e. sum of observed brood sizes/ number of nest checks) and sex of the nestling were designated as additional covariates, as they may also affect growth. Since brood size may change over time due to death from predation or starvation (e.g. brood reduction is common in red-winged blackbirds; Forbes & Glassey 2000), I used mean brood size to account for these changes. To identify variables that had a significant effect on nestling growth, I used stepwise backwards selection in which variables with the highest p-value were sequentially removed from the mixed effects model until only variables with significant effects remained. I report the probability values of each significant variable as well as the focal variable, i.e. blood THg concentration. To further understand the relationship between blood THg concentrations and body growth, I performed a Pearson's correlation test (cor.test- function).

RESULTS

Mercury Concentrations

Mean levels of blood THg in adults were significantly higher than nestlings (Table 1). There was no significant difference between nestling THg concentrations at Kenridge Farm and the Bronx River (Table 2). Adult blood THg concentrations also did not differ

between locations (Table 2). Mean mercury levels were not significantly different between adult males and females (Table 1).

Nestling growth Rates

The PCA of these parameters produced three eigenvectors. The first eigenvector (PC1), which encompassed 67 % of the variation in growth measurements (Table 3), represented nestling body growth. All measurements had positive factor loadings (Table 3). Thus, high PC1 scores indicated fast body growth, whereas low PC1 scores indicated slow body growth.

Blood THg levels showed no significant effect on body growth ($t = 0.502$, df = 21, $p = 0.621$) (Figure 1). The only variable to have a significant effect on body growth was the sex of the nestling ($t = 4.131$, df = 23, $p < 0.001$).

There was no relationship between blood THg concentrations and nestling body growth (PC1) (Pearson's correlation coefficient: $r = 0.153$; $t = 0.9394$, df = 37, $p = 0.354$; Figure 1).

DISCUSSION

Mercury concentrations

The findings from this study suggest that red-winged blackbird populations nesting in the New York metropolitan area are exposed to mercury at levels typically found in insectivorous passerine populations in the northeastern United States (Evers et al. 2005). Blood mercury concentrations fell within ranges seen in both aquatic (e.g. tree swallow, *Tachycineta bicolor*) and terrestrial feeding species (e.g. Bicknell's thrush, *Catharus*

bicknelli) (Driscoll et al. 2007), which is indicative of the species' broad dietary preferences. Also, adult levels were intermediate of those reported in red-winged blackbirds from other locations. Mercury concentrations exceeded levels of individuals nesting in the New Jersey Meadowlands (Tsipoura et al. 2008), but were less than values recorded from individuals in Massachusetts (Evers et al. 2005).

I found no significant differences in blood mercury concentrations between locations. This result may simply be a product of small sample size in which there was not enough statistical power to resolve if a difference truly existed, or both sites may in fact have similar levels of mercury contamination. Two reasons can possibly explain the latter. Because river systems tend to accumulate mercury less readily due to their ability to continually flush contaminants (Evers et al. 2005), contamination levels in the riparian habitat of the urban site may have remained low and therefore, were more comparable to that of the suburban site. On the other hand, the freshwater pond habitat of the suburban site may have acted as a terminal basin for persistent mercury accumulation. This in turn caused exposure levels to reach those of the urban site.

Thus, habitat types vary in their ability to accumulate mercury (Evers et al. 2005). Drought and flooding can also influence contaminant levels (Brasso & Cristol 2008; Gerrard & St. Louis 2001); and so, more research is needed to resolve the levels of mercury exposure across populations and their possible environmental drivers.

Dietary differences due to differences in available prey items may have also caused individuals in the suburban site to exhibit mercury levels similar to that of the urban site. Adults and nestlings at Kenridge Farm were observed feeding more often on predatory invertebrate prey, like dragonflies (order Odonata; pers. obs. Allisyn Gillet),

that occupy higher trophic positions. As species higher in the food chain are able to bioaccumulate and biomagnify mercury, predatory invertebrates within a low contamination environment could harbor high contaminant loads and expose insectivorous birds to mercury levels typically found in more polluted environments (Cristol et al. 2008; Rimmer et al. 2010). Future research exploring the variation in diet or available prey (i.e. invertebrate species abundance) between habitat types is needed.

Mercury exposure did not vary across sexes. This was unexpected as females are able to deposit organic mercury in their eggs (Heinz et al. 2009). However, Evers et al. (2005) explain that MeHg loss may be compensated through dietary intake within weeks of egg laying.

Blood mercury was significantly lower in nestlings than adults. This is most likely due to the sequestration of mercury in feathers during development, as mercury has a tendency to bond to sulfhydryl groups in keratin (Tsipoura et al. 2008). Mercury sequestration during feather growth can then protect the nestling from harmful physiological effects (Kenow et al. 2003), but this has yet to be determined, as nestlings may be more sensitive to low concentrations of mercury than previously assumed (Franceschini et al. 2009; Gariboldi et al. 1998).

Adults and nestlings exhibited blood mercury concentrations well below impact thresholds of common loon (*Gavia immer*) adults and bald eaglets (*Haliaeetus leucocephalus*) (3.0µg/g w.w. and 1.0 µg/g w.w., respectively) (Evers et al. 2007). However, since sublethal effects are hard to detect (Rimmer et al. 2010), it is difficult to ascertain whether or not red-winged blackbirds were adversely impacted. Tolerance levels to mercury exposure vary across species (Braune 1987; Heinz et al. 2009); and so,

I cannot confidently infer from these thresholds that the red-winged blackbird populations in this study experienced no harmful effects.

To my knowledge, impact thresholds have not been determined in passerines. Seewagen (2009) suggests that threshold levels in passerines may be lower than those established in other well-researched species. As nestlings may be more sensitive to exposure than adults (Franceschini et al. 2009; Gariboldi et al. 1998), it remains a research priority to not only establish threshold levels in passerines, but also those that recognize differences within and across species (Seewagen 2009).

Nestling growth rates

I did not find a relationship between nestling blood mercury concentrations and growth. I assume that the sequestration of mercury in feathers (Condon & Cristol 2009) prevented blood mercury concentrations from reaching levels that adversely affected nestling growth seen in previous studies (Fimreite & Karstad 1971; Spalding et al. 2000). Common loon nestlings administered daily doses of 1.5 μg/g of MeHg (i.e. three times the typical level of exposure) from the ages day 7 to day 112 showed no significant effects of mercury on growth (Kenow et al. 2003). By the 105th day, nestling feathers harbored 26% of mercury body burden. Furthermore, Kenow et al. (2003) observed rapid declines in blood mercury as feathers grew, but increases in concentrations once growth ended.

Two populations of tree swallows in Maine exhibited a negative effect of feather mercury on the linear growth rate of nestling weight (Longcore et al. 2007a). However, the authors did not observe any significant relationships between feather mercury and

more stable morphological measurements, like tarsus length and wing chord. Captive great egret chicks (*Ardea albus*) fed 0.135mg/kg MeHg daily similarly experienced no adverse effects until week 9, when feathers stopped growing (Spalding et al. 2000). However, after the cessation of feather growth, chicks exhibited reduced appetite and weight loss.

Although it may seem that nestlings are naturally protected from high mercury exposure, they are at greatest risk when feather growth ends. Spalding et al. (2000) note that the cessation of feather growth normally occurs during fledging at which the individual may be at highest risk to predation and disease. Thus, it is crucial that research continues to explore the toxicological effects of mercury in young birds.

Conclusions

In this study, there was no significant difference in mercury exposure levels between an urban and a suburban population of red-winged blackbirds. Because mercury accumulates differentially among habitat types and invertebrate prey items, this finding may have been a result of environmental factors. Mercury levels also did not vary between males and females. However, adults exhibited significantly higher levels of mercury in their blood than nestlings. Lower levels of blood THg concentrations in nestlings may have occurred due to the sequestration of mercury in growing feathers. Overall, levels of mercury exposure between both populations were similar to levels previously reported in red-winged blackbirds and other insectivorous passerines found in the northeastern United States. Finally, I did not find a significant relationship between nestling growth and levels of mercury exposure, as mercury concentrations may have been too low to resolve a significant effect. This study bolsters the hypothesis that

nestlings are naturally protected from high mercury exposure during development, but more research is needed to resolve the potential adverse impacts after feather growth cessation.

Table 1: Mean THg concentrations (± S.E. mg/kg, wet weight) separated by age and sex

			F	d.f.	*p*
Age	Adult	Nestling			
	0.173 ± 0.051 (14) [a]	0.057 ± 0.006 (59) [a]	18.3	1, 71	< 0.001*
Sex	Male [b]	Female [b]			
	0.120 ± 0.032 (7) [a]	0.226 ± 0.096 (7) [a]	1.095	1, 12	0.316

[a] Values represented in parentheses represent sample sizes for each group.

[b] corresponds to adults only

[*] statistically significant difference

Table 2: Mean THg concentrations (± S.E. mg/kg, wet weight) separated by location

	Bronx River	Kenridge Farm	*F*	d.f.	*p*
Adult	0.061 ± 0.016 (5) [a]	0.234 ± 0.071 (9) [a]	3.183	1, 12	0.100
Nestling	0.040 ± 0.012 (8) [a]	0.060 ± 0.006 (51) [a]	1.453	1, 57	0.233

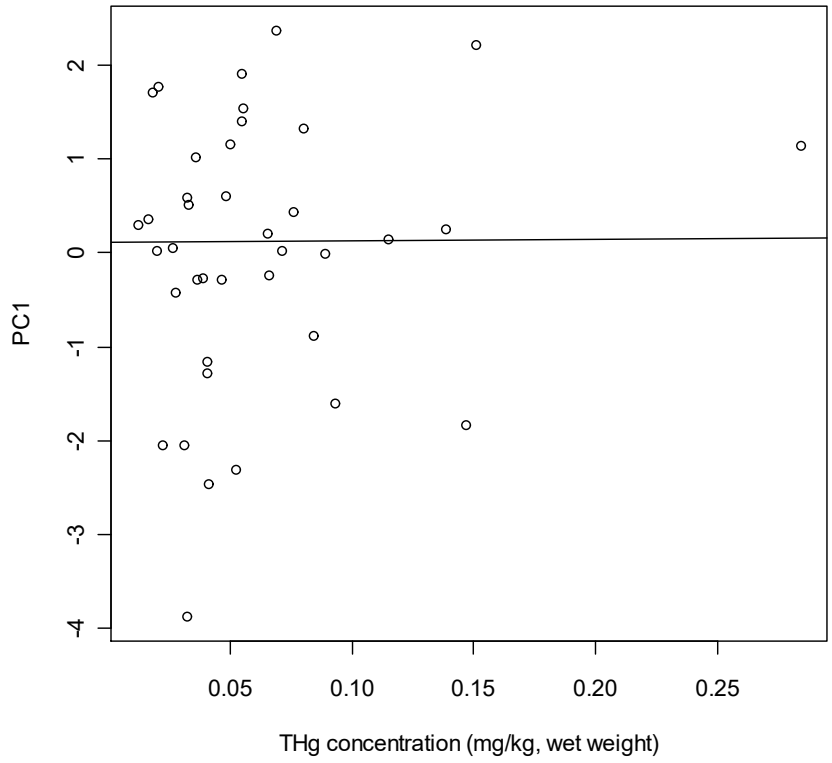
[a] Values represented in parentheses represent sample sizes for each category.

Table 3: Principal components analysis on nestling morphological measurements

	PC1	PC2	PC3
Mass	0.601	-0.467	-0.649
Tarsus length	0.633	-0.218	0.743
Wing chord	0.488	0.857	-0.165
% variance[a]	67	23	9

[a] This value represents the percent of variance that is explained by each eigenvector (i.e. principal component).

Figure 1: The relationship between the rate of nestling body growth and blood THg concentrations exhibits a weak positive correlation that is not significant (Pearson's correlation coefficient: $r = 0.153$; $t = 0.9394$, df = 37, $p = 0.354$).



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