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Avian Behavior, Ecology, and Evolution

Eggshell thickness and egg morphometrics in five songbird species from the Central Valley, California

Grosor de la cascara y morfometría de huevos en cinco especies de aves canoras del Valle Central, California

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ABSTRACT. Avian eggshell thickness is an important life history metric in birds and has broad applications across disciplines ranging from animal behavior to toxicology. Empirical eggshell thickness values for songbirds (Order Passeriformes) are under-represented in the literature due to the difficulty of measuring smaller eggs using traditional methods. We used a Hall-effect thickness gauge to measure eggs of five focal songbird species from California's Central Valley: House Wren (*Troglodytes aedon*; *n* = 567), Tree Swallow (*Tachycineta bicolor*; *n* = 297), Ash-throated Flycatcher (*Myiarchus cinerascens*; *n* = 21), Western Bluebird (*Sialia mexicana*; *n* = 13), and Bewick's Wren (*Thryomanes bewickii*; *n* = 5). We compared minimum eggshell thickness measurements at the equator and sharp pole, and we related eggshell thickness to other egg morphometrics and adult body mass. Eggshell thickness at the equator was 5.6% thicker in Ash-throated Flycatchers and 3.5% thinner in Tree Swallows compared with eggshell thickness at the sharp pole. Among species, eggshell thickness at the sharp pole was greater in species with larger eggs, whereas, within species, larger eggs were thinner at the sharp pole. Eggshells were 8% and 11% thinner in late incubation eggs (≥75% of total incubation duration) than early incubation (≤10% of total incubation duration) for House Wren and Tree Swallow eggs, respectively. Whenever possible, it is preferable to use empirical eggshell thickness data that are specific to the species and geographic region being studied, and a relatively new method used in this study allows accurate measurement of small eggs without having to compromise the integrity of preserved eggshell specimens.

RESUMEN. El grosor de la cáscara de huevo en aves es una medida importante en la historia de vida de las aves y tiene aplicaciones amplias en disciplinas que van desde el comportamiento animal hasta la toxicología. Los valores empíricos del grosor de la cáscara de huevo para aves canoras (Orden Passeriformes) están subrepresentados en la literatura debido a la dificultad de medir huevos más pequeños mediante métodos tradicionales. Utilizamos un medidor de grosor por efecto Hall para medir huevos de cinco especies de aves canoras del Valle Central de California: *Troglodytes aedon* (n = 567), *Tachycineta bicolor* (n = 297), *Myiarchus cinerascens* (n = 21), *Sialia mexicana* (n = 13), y *Thryomanes bewickii* (n = 5). Comparamos las mediciones mínimas de grosor de la cáscara entre el ecuador y el polo agudo de los huevos, y relacionamos el grosor de la cáscara de huevo con otras medidas morfométricas del huevo y la masa corporal del adulto. El grosor de la cascara en el ecuador fue un 5,6% más grueso en *M. cinerascens* y un 3,5% más delgado en *T. bicolor* en comparación con el grosor de la cascara en el polo agudo. Entre especies, el grosor de la cáscara de huevo en el polo agudo fue mayor en especies con huevos más grandes, mientras que, dentro de las especies, los huevos más grandes fueron más delgados en el polo agudo. Las cáscaras de huevo fueron un 8% y un 11% más delgadas cuando los huevos se encontraban en etapas avanzadas de la incubación (≥75% de la duración total de la incubación) que cuando se encontraban en etapas tempranas (≤10% de la duración total de la incubación) en el caso del *T. aedon* y *T. bicolor*, respectivamente. Siempre que sea posible, es preferible utilizar datos empíricos del grosor de la cáscara de huevo que sean específicos para la especie y la región geográfica de estudio, y un método relativamente nuevo utilizado en este estudio permite obtener mediciones precisas de huevos pequeños sin comprometer la integridad de las cáscaras de huevo preservadas como muestras.

Key Words: *egg morphometrics; eggshell thickness; eggshell thinning; embryonic development; Passeriformes; passerine; songbird*

INTRODUCTION

Avian eggshell thickness has important implications for gas exchange and water loss between eggs and the environment (Ar et al. 1974, Ar and Rahn 1985, Stein and Badyaev 2011), structural integrity and protection (Spaw and Rohwer 1987, Picman et al. 1996), and studies of environmental contamination (Ratcliffe 1970, Fair and Myers 2002). Eggshell thickness can be influenced by a variety of ecological and environmental factors, including evolutionary pressures (Spaw and Rohwer 1987, Picman et al. 1996), changes that occur during embryonic development (Castilla et al. 2010, Orłowski and Hałupka 2015), and exposure to contaminants (Hickey and Anderson 1968, Cooke 1973). As an example, some species have thicker eggshells than would be predicted based on bird body size, either to resist predation or to facilitate brood parasitism by making eggs more resistant to puncture and ejection from the nest (Spaw and Rohwer 1987, Picman et al. 1996). The availability and application of eggshell measurements have substantial implications for ecotoxicology research on contaminant concentrations in eggs. A lack of precise eggshell thickness measurements influences estimates of egg contaminant concentrations because the calculations require accurate estimates of egg content volume, which is generally calculated based on fresh egg weight and egg dimensions. Specifically, Herzog et al. (2016) determined that failing to account for eggshell thickness in calculations led to a 6–13% underestimation of contaminant concentrations in egg contents.

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Empirical data on songbird (Passeriformes) eggshell thickness for use in ecological or toxicological studies are under-represented in the literature. This is possibly due to difficulty in measuring small eggs compared with large eggs using older techniques. Newer methods for measuring eggshell thickness may provide more precise and accurate measurements than prior approaches and allow for measurement of smaller eggs and at more locations on the eggshell. Hall-effect thickness gauges measure the distance between a small steel reference ball rolled along the inner surface of an eggshell and a magnetic probe (Santolo 2018). This method is accurate to 0.001 mm, with less human error and higher repeatability than analog thickness gauges (Santolo 2018). It also allows for the eggshell to remain largely intact, even for smaller and more fragile eggs, as well as allowing for measurements to be taken across the surface of an intact eggshell rather than just in those areas accessible from the blow hole that is used to remove egg contents during specimen preservation (Santolo 2018). Traditional thickness gauges or micrometers, on the other hand, either require destruction of eggshell specimens to access multiple locations on the eggshell or restrict measurement to the area adjacent to or across from the blow hole (as in the case of museum specimens). Another common approach in the literature is to use morphometric measurements to calculate an index of eggshell thickness (Ratcliffe 1970, Ar et al. 1974, Maurer et al. 2012), or rely on existing tables of eggshell thickness estimates, many of which are themselves based on estimated values rather than empirical measurements (Schönwetter and Meise 1960, Maurer et al. 2010). The limitation of older methods is especially evident among songbird species with small eggs. Empirical eggshell thickness values for these species, when they are available, are usually limited to measurements along the equator of the egg (widest point) due to the difficulties involved in preparing and measuring the sharp pole (the more pointed end of the egg opposite to where the air cell forms; Orłowski et al. 2016). In some species, eggshell thickness may vary among locations on the egg (Orłowski et al. 2019*b*; Peterson et al. 2020). We found only one study specifically reporting songbird eggshell thickness at the sharp pole of the egg, which they found to be significantly thinner than other regions of the egg (Gosler et al. 2005), and one study that measured sharp pole thickness for an examination of shell thinning during embryonic development (Ding et al. 2019).

We used a Hall-effect thickness gauge (Santolo 2018) to measure multiple eggs from five species of songbirds from California's Central Valley: Ash-throated Flycatcher (*Myiarchus cinerascens),* Bewick's Wren (*Thryomanes bewickii*), House Wren (*Troglodytes aedon*), Tree Swallow (*Tachycineta bicolor*), and Western Bluebird (*Sialia mexicana*). Our objectives were to (1) report eggshell thickness data for these species, (2) compare eggshell thickness at the sharp pole of the egg (rarely measured in previous studies) to the equator (more commonly reported), (3) relate eggshell thickness to other egg morphometric measurements and determine if predictive equations could be derived to allow estimates of eggshell thickness for other songbird species, and (4) evaluate how our empirical data for songbirds fit into predictive equations that were derived using similar data for non-songbird species.

METHODS

Sample collection

We collected eggs at two sites (Cache Creek Settling Basin: 38.7° N, 121.7°W and Cosumnes River Preserve: 38.3°N, 121.4°W) in California's Central Valley from 2014 to 2018 as part of studies on avian ecology and contaminant exposure. Collected eggs were either abandoned before hatch, unhatched eggs from clutches where other eggs hatched, viable eggs sampled randomly (one per nest), or from nests where the entire clutch was collected for studies on maternal transfer of contaminants (Ackerman et al. 2017). The adult birds were not marked; thus, it is unknown if the eggs were from the first or subsequent breeding attempts. When collected, eggs were placed in coolers with wet ice in the field and then stored in a 2°C refrigerator until processing. Individual nests and eggs were assigned unique identifiers.

Egg processing

First, we cleaned the exterior egg surface with deionized water and isopropyl alcohol and allowed the egg to dry. We measured egg length (±0.01 mm) and width (±0.01 mm) using digital calipers (Mitutoyo, Aurora, Illinois, USA) and whole egg mass (±0.01 g) using a digital scale (Ohaus Adventurer® Pro AV212, Ohaus Corporation, Parsippany, New Jersey, USA). We then dissected the egg, using stainless steel scissors and tweezers to cut a circle approximately 5 mm in diameter at the blunt end. Egg contents were removed into jars, and most (869/907, 96%) embryos were aged to the nearest half day (Hamburger and Hamilton 1951, Hemmings and Birkhead 2016). After dissection, eggshells were stored in a -20°C freezer.

Before further processing, dissected eggshells were removed from the freezer and allowed to warm to room temperature. We then rinsed the inside of the eggshells with a mild detergent (1% Alconox, Alconox, Inc., White Plains, New York, USA) and used a cotton swab to wipe the surface and remove any remaining contents, leaving the eggshell membrane intact. If necessary, a small stainless-steel spatula was used to gently dislodge egg contents that remained adhered to the inside of the eggshell. We then rinsed the eggshell several times using deionized water. After cleaning, eggshells were dried in a drying oven for 24 h at 40°C and then stored in a desiccator until they were measured.

Eggshell thickness measurements

We measured the minimum eggshell thickness of each egg at both the equator and the sharp pole, with the exception of 36 eggs for which the sharp pole could not be measured. The blunt pole, where the air cell develops, was not measured because of how the eggs were opened up during dissection. We measured eggshell thickness using a Magna-Mike® 8600 Hall-effect thickness gauge (Olympus Scientific Solutions Americas Corporation, Center Valley, Pennsylvania, USA) with a 1.58 mm steel measurement ball. We calibrated the thickness gauge at the start of each day and any time the machine was idle for more than 1 h. Following established protocols (Santolo 2018, Peterson et al. 2020) to obtain the minimum eggshell thickness, we rolled the magnetic measurement ball across the inner surface of the egg, which included the outer eggshell membrane attached to the eggshell. At the equator, we ensured that the whole surface was sampled by slowly rotating the measurement ball within the egg for three to five complete revolutions inside the egg, covering the whole equator when possible, or back and forth over as much of the equator as possible if the eggshell was not completely intact (damaged during dissection). This instrument and method provide a minimum eggshell thickness across the surface of an eggshell, which we captured as the measurement ball was rolled across the inner surface of the eggshell. At the sharp pole, we rolled the measurement ball around a 1–2 mm circle inside the egg to cover the whole pointed end of the egg and recorded the minimum measurement that was obtained at the sharp pole. Pigmented spots (maculation) on eggshells can be thinner than plain sections of eggshells (Gosler et al. 2005). We rolled the measurement ball across plain and maculated portions (pigment spots) of eggshells to capture the thinnest measurement; therefore, the measurements may reflect pigmented sections of Ash-throated flycatcher, Bewick's Wren, and House Wren eggs, whereas Tree Swallow and Western Bluebird eggs are uniformly colored. Irrespective of whether eggs were maculated or not, our method was consistent among species and captured the thinnest measurement of eggshells.

Statistical analysis

Eggshell thickness among species and measurement location

We compared eggshell thickness measured at the sharp pole to eggshell thickness measured at the equator using a linear mixed effect model (lme4 package; Bates 2015), with measurement location (sharp pole or equator) and species as fixed effects, and egg identification nested within nest identification as a random effect to account for times when more than one egg was sampled from the same nest. We used the Kenward-Rogers approximation for degrees of freedom and *F* tests for significance (afex package; Singmann et al. 2020). We then used model-generated least squares means to make pairwise comparisons between measurement locations within each species, and among species at the same measurement location (emmeans package; Lenth 2020). Residuals from these analyses and all subsequent models were examined to verify that the assumptions of linear regression were met.

Within-eggshell relationships between the thickness at the sharp pole and the equator

To determine the slope of the relationship between eggshell thickness at the sharp pole and at the equator within species, using individual eggshell measurements, we used a general linear model with equator thickness and species as fixed effects, and the interaction between equator thickness and species (car package; Fox and Weisberg 2019). Next, we tested the linear relationship between sharp pole thickness and equator thickness among species using a weighted regression on the species mean values for the equator and sharp pole thickness measurements. We used the natural log of the sample size for each species to weight the model, so that species with more samples were weighted more than species with fewer samples (Peterson et al. 2020). Due to the small number of species represented, we conducted randomization tests to calculate *p* values based on the actual distribution of the data for all species comparisons of mean eggshell thicknesses (5,000 iterations; Edgington 1964).

Eggshell thickness vs. morphometrics and adult body mass

We examined the relationships between eggshell thickness (separate models for equator and sharp pole thickness), egg morphometric measurements, and average adult female body mass. Egg morphometrics included egg length, egg width, and egg volume. Egg volume (mL) was calculated using the equation from Hoyt (1979): egg volume = K_v × egg length × egg width², where K_v is an egg shape coefficient. For the among-species regression with species mean female body mass, we used the mean body mass of adult females captured in California's Central Valley 2012–2013 as temporally close as possible to the time of egg collection (Ackerman et al. 2019), except for the Western Bluebird, for which we used the adult female mass reported by Dunning (2008). For comparison with egg morphometric measurements within species using individual eggshell measurements, we used general linear models that included terms for the morphometric measurement in question, species, and the interaction between morphometric measurement and species. Among species, we used general linear models to conduct weighted regressions on the species mean values for eggshell thicknesses and morphometric measurements (weighted using the natural log of the sample size). We $\log_e$-transformed adult mass before analysis because we did not expect bird mass to scale linearly with eggshell thickness (Birchard and Deeming 2009).

Eggshell thickness for early and late-stage eggs

For comparisons of embryo age (within species only) between eggs early and late in incubation, we first calculated relative incubation age for each egg (embryo age determined during dissection / average duration of incubation) to standardize incubation age, as described in Peterson et al. (2020). We used Welch's t-tests to compare eggshell thickness at the equator between eggs early in incubation (<10% of total incubation) and late in incubation (>75% of total incubation) for the two species (House Wren and Tree Swallow) for which we had at least three eggs from both time periods. We selected 75% to identify late incubation eggs because prior observations in Capercaillie (*Tetrao urogallus*) suggested eggshell thinning occurred in the final quarter of the incubation period (Orłowski et al. 2019*a*).

Allometric models of eggshell thickness

We initially calculated the predicted eggshell thickness values for our passerine species using linear equations from Peterson et al. (2020) for sharp pole thickness vs. equator thickness and for equator thickness vs. egg length. However, initial plots showed that the passerine data did not fit into a linear relationship with the non-passerine data. Therefore, we developed new allometric models to predict the among-species relationship between sharp pole thickness and equator thickness as well as to predict equator thickness from egg length. In both cases, we created a set of four models that increased in complexity from a simple linear model, quadratic model, cubic model, as well as a more standard allometric relationship of the log-log model. All models were run using all available data, including the data from Peterson et al. (2020), and we compared models using Akaike Information Criterion corrected for small sample sizes (AIC_c). The most supported model (model weight = 0.95) to predict species mean sharp pole eggshell thickness from equator thickness was the log-log model. All other models had a ΔAIC_c >7.1 and model weight <0.03. Similarly, the most supported model (model weight = 0.99) to predict species mean eggshell equator thickness from egg length was the log-log model. All other models had ΔAIC_c >14.8 and model weight <0.001. Therefore, in the results we will report results from the log-log models and show the improved fit over the previously described linear models.

Table 1. Arithmetic mean ± SD eggshell thickness and egg morphometric measurements (range) for songbird (Passeriformes) eggs from California's Central Valley (2014–2018).

	Number of eggs	Egg length (mm)	Egg width (mm)	Egg volume (mL)	Equator sample size	Eggshell thickness at equator (mm)	Sharp pole sample size	Eggshell thickness at sharp pole (mm)	Mean adult female body mass (g)[a]
House Wren	567	16.5 ± 0.7 (14.2-18.6)	12.5 ± 0.4 (11.0-14.1)	1.3 ± 0.1 (0.9-1.8)	567	0.080 ± 0.008 (0.049-0.112)	549	0.080 ± 0.010 (0.054-0.123)	10.5
Tree Swallow	297	18.4 ± 0.9 (16.1-20.6)	13.0 ± 0.4 (11.4-14.2)	1.6 ± 0.1 (1.1-2.0)	297	0.080 ± 0.008 (0.046-0.114)	282	0.083 ± 0.011 (0.043-0.120)	16.6
Ash-throated Flycatcher	21	22.4 ± 1.1 (19.9-24.7)	16.8 ± 0.4 (15.8-17.7)	3.2 ± 0.2 (2.6-3.6)	21	0.098 ± 0.008 (0.085-0.115)	21	0.093 ± 0.009 (0.079-0.115)	28.6
Western Bluebird	13	21.0 ± 0.6 (20.0-22.0)	15.9 ± 0.5 (15.0-17.2)	2.7 ± 0.2 (2.4-3.2)	13	0.086 ± 0.007 (0.075-0.096)	13	0.089 ± 0.011 (0.061-0.102)	27.1
Bewick's Wren[b]	5	16.8 ± 0.8 (15.5-17.4)	12.9 ± 0.6 (12.3-13.8)	1.4 ± 0.2 (1.1-1.6)	5	0.079 ± 0.009 (0.066-0.087)	3	0.079 ± 0.010 (0.070-0.089)	9.3

[a] Adult female body masses of songbirds in the Central Valley during the same time period (Ackerman et al. 2019), except for the Western Bluebird, for which we used the adult female mass reported in Dunning (2008).
[b] Not included in the regression analyses that used individual egg morphometrics due to smaller sample sizes.

All analyses were performed using R v. 4.1.2 (R Core Team 2020). For statistical analyses, we included all five species in among-species comparisons of mean values and among-species regression analyses, but we excluded Bewick's Wren (n = 3 measurements at the sharp pole, n = 5 at the equator) from within-species regressions.

RESULTS

We measured eggshell thickness of 903 eggs from five songbird species (Table 1; raw data in Peterson and Ackerman 2024). The relative incubation age of collected eggs ranged from 0–93% of total incubation duration but were mostly younger eggs, with 61% of eggs in the analysis <10% through the incubation period, and 85% of eggs in the analysis <25% through the incubation period. At the equator, the thickest individual eggshells within a species were 28–148% thicker than the thinnest eggshells, demonstrating variability in eggshell thickness within species. At the sharp pole, the thickest individual eggshells within a species were 27–179% thicker than the thinnest eggshells.

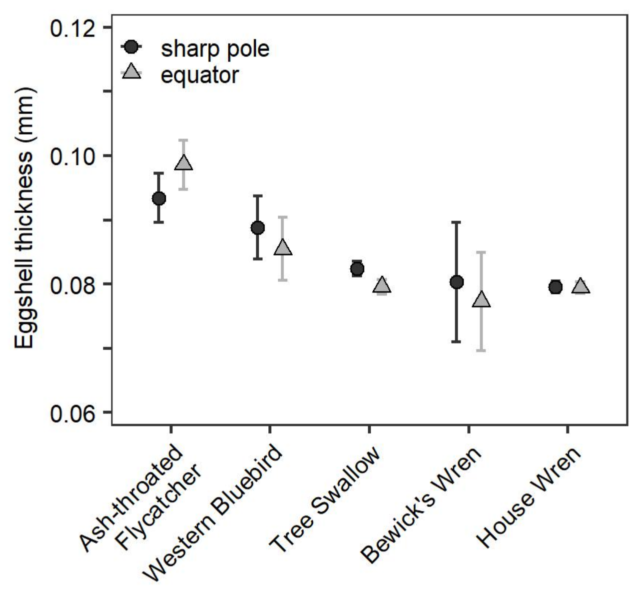
Eggshell thickness among measurement locations and species

The global model showed a significant interaction between measurement position on the egg (equator vs. sharp pole) and species ($F_{4,907.4}$ = 7.6, p < 0.001), so we used least squares means to make pairwise comparisons among the five species and between sharp pole and equator thickness within each species.

Within species, we detected no difference between equator thickness and sharp pole thickness for Bewick's Wren, House Wren, and Western Bluebird (all $t \leq 1.4$, all $p \geq 0.16$; Fig. 1). Ash-throated Flycatcher eggs were 5.6% thicker at the equator than at the sharp pole ($t_{865.3}$ = 2.7, p = 0.007; Fig. 1). Tree Swallow eggs were 3.5% thinner at the equator than at the sharp pole ($t_{882.6}$ = 5.6, p < 0.001; Fig. 1).

Among species, Ash-throated Flycatcher and Western Bluebird eggs were significantly thicker at the equator than House Wren eggs (Ash-throated Flycatcher: 24.2% thicker, Western Bluebird: 7.6% thicker) and Tree Swallow eggs (Ash-throated Flycatcher: 23.9% thicker, Western Bluebird: 7.4% thicker; all $t \geq 2.3$, all p < 0.022; Fig. 1). Ash-throated Flycatcher and Western Bluebird eggs were also significantly thicker at the sharp pole than House Wren eggs (Ash-throated Flycatcher: 17.4% thicker, Western Bluebird: 11.7% thicker) and Tree Swallow eggs (Ash-throated Flycatcher: 13.3% thicker, Western Bluebird: 7.7% thicker; all $t \geq 2.5$, all $p \leq$ 0.013). Bewick's Wren eggs were significantly thinner than Ash-throated flycatcher eggs at both measurement positions (21.6% thinner at equator, 14.0% thinner at sharp pole: all $t \geq 2.6$, all $p \leq$ 0.011).

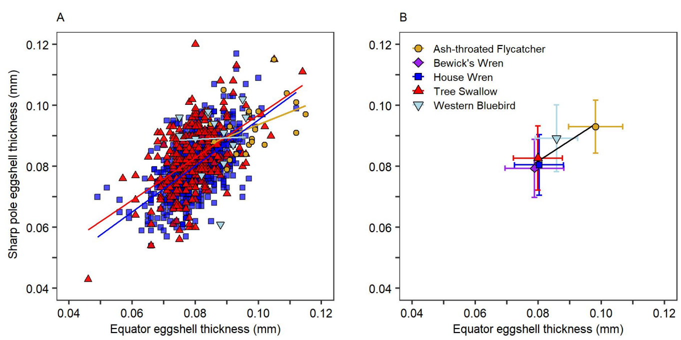
Fig. 1. Model-derived least squares mean eggshell thickness (with 95% confidence intervals) at two egg measurement locations of songbird eggs from California's Central Valley (2014–2018).



Within-eggshell relationships between the thickness at the sharp pole and equator

There was no detectable interactive effect of species ($F_{3,857}$ = 2.0, p = 0.11) on the relationship between sharp pole eggshell thickness and equator eggshell thickness. Sharp pole thickness and equator thickness were positively related (adjusted R^2 = 0.34, $F_{1,857}$ = 382.5, p < 0.001; Fig. 2*a*; Table 2), after accounting for species ($F_{3,857}$ = 7.2, p < 0.001). Of the two species for which the previous model did not detect a within-species difference in least squares means between measurement positions, there was a strong positive relationship between sharp pole thickness and equator thickness within individual House Wren eggs (slope = 0.75; Fig.2*a*), whereas we found little or no relationship between measurement positions in Western Bluebird eggs (slope =0.05; Fig. 2*a*). Tree Swallow eggs, which were, on average, thinner at the equator than at the sharp pole, showed a strong positive relationship between eggshell thickness at the two measurement positions (slope = 0.70; Fig. 2*a*). Ash-throated Flycatcher eggs, which were thicker at the equator than at the sharp pole, also showed a positive relationship between measurement positions within eggs, albeit with a shallower slope (slope = 0.41; Fig. 2*a*).

Fig. 2. Eggshell thickness at the sharp pole related to eggshell thickness at the equator in songbird eggs collected from California's Central Valley 2014–2018. (A) Individual eggshell measurements with slopes for each species extracted from the global model. (B) Mean (± SD) eggshell thickness (raw data) for each species shown with the among-species regression line on mean values.



There was a significant positive relationship between species mean sharp pole thickness and species mean equator thickness based on a regression with just these five songbird species (adjusted R^2 = 0.82, $F_{1,3}$ = 19.3, p = 0.03; Fig. 2*b*).

Eggshell thickness vs. egg morphometrics

Based on the analysis including all individual egg measurements, we detected a negative relationship between sharp pole thickness and egg length ($F_{1,856}$ = 40.9, p < 0.001; Fig. 3*a*; Table 2), after accounting for differences among species ($F_{3,856}$ = 27.0, p < 0.001), and a non-significant interaction between egg length and species ($F_{3,856}$ = 0.6, p = 0.61). We also detected a negative relationship between sharp pole thickness and egg volume ($F_{1,856}$ = 4.3, p = 0.039; Fig. 4*a*; Table 2), after accounting for species ($F_{3,856}$ = 5.6, p < 0.001), and a non-significant interaction between egg volume and species ($F_{3,856}$ = 0.3, p= 0.80). In contrast, we found no relationship between sharp pole eggshell thickness and egg width ($F_{1,857}$ = 1.8, p = 0.18; Fig. 5*a*), after accounting for species ($F_{3,857}$ = 1.5, p = 0.21) and a non-significant interaction between egg width and species ($F_{3,857}$ = 0.2, p = 0.89). After accounting for the effect of species (all F > 15.0, all p < 0.001), we found no significant

Table 2. Slope and intercept values for individual species (with sample size >10 eggs) extracted from global models testing the relationship between eggshell thickness at the sharp pole and at the equator, and between sharp pole eggshell thickness and various egg morphometric measurements.

Species	Slope
Sharp pole (mm) ~ equator (mm)	
Ash-throated Flycatcher	0.411 × equator eggshell thickness + 0.053
House Wren	0.754 × equator eggshell thickness + 0.020
Tree Swallow	0.698 × equator eggshell thickness + 0.027
Western Bluebird	0.051 × equator eggshell thickness + 0.085
Sharp pole (mm) ~ egg length (mm)	
Ash-throated Flycatcher	-0.002 × egg length + 0.144
House Wren	-0.003 × egg length + 0.138
Tree Swallow	-0.002 × egg length + 0.126
Western Bluebird	0.000 × egg length + 0.092
Sharp pole (mm) ~ egg volume (mL)	
Ash-throated Flycatcher	-0.002 × egg volume + 0.100
House Wren	-0.008 × egg volume + 0.091
Tree Swallow	-0.004 × egg volume + 0.089
Western Bluebird	-0.007 × egg volume + 0.082

relationships between equator eggshell thickness and egg morphometrics within species (egg length: $F_{1,889}$ = 0.1, p = 0.72, Fig. 3*c*; egg width: $F_{1,890}$ = 0.2, p = 0.63, Fig. 5*c*; egg volume: $F_{1,889}$ = 0.5, p = 0.47, Fig. 4*c*).

Among species, we observed strong positive relationships between species mean sharp pole thickness and species mean egg length ($F_{1,3}$ = 95.4, p < 0.01; slope = 0.002, intercept = 0.044; Fig. 3*b*), species mean egg width ($F_{1,3}$ = 111.8, p < 0.01; slope = 0.003, intercept = 0.046; Fig. 5*b*), and species mean egg volume ($F_{1,3}$ = 299.6, p < 0.001; slope = 0.006, intercept = 0.072; Fig. 4*b*). We also found positive relationships between species mean equator thickness and species mean egg length ($F_{1,3}$ = 9.8, p = 0.03, slope = 0.003, intercept = 0.034; Fig. 3*c*), species mean egg width ($F_{1,3}$ = 15.3, p = 0.04, slope = 0.004, intercept = 0.033; Fig. 5*c*), and species mean egg volume ($F_{1,3}$ = 17.7, p = 0.04, slope = 0.008, intercept = 0.068; Fig. 4*c*). Among the five species, mean egg length ranged from 16.5 mm to 22.4 mm (35.8% difference), resulting in a predicted increase of 0.013 mm (15.9% difference) in sharp pole eggshell thickness between the shortest and longest species mean egg length represented in this study.

Eggshell thickness vs. adult mass

Mean adult female body masses in the five species of songbirds we analyzed ranged from 9.3–28.6 g, a 207.5% difference between the smallest and largest species. We detected a positive relationship between species mean eggshell thickness at the sharp pole and $\log_e$-transformed species mean adult female body mass ($F_{1,3}$ = 24.6, p = 0.03, slope = 0.011, intercept = 0.054; Fig. 6) but we did not detect the same relationship at the equator ($F_{1,3}$ = 4.4, p =0.08).

Eggshell thickness for early and late-stage eggs

Eggshell thickness at the equator of eggs late in incubation, within the final quarter of the incubation duration (n = 6), were 7.9% thinner in House Wren (t = 6.12, df = 7.4, p < 0.001) than eggs

Fig. 3. Eggshell thickness at the sharp pole and at the equator as a function of egg length within and among songbird species from California's Central Valley 2014–2018. (A) Sharp pole eggshell thickness of individual eggs decreased as a function of egg length after accounting for differences among species, with slopes for each species extracted from the global model. (B) Species-specific arithmetic mean (± SD) eggshell thickness at the sharp pole increased with species mean egg length (raw data), shown with the among-species regression line. (C) Based on individual eggs, we did not detect a significant relationship between eggshell thickness at the equator and egg length, after accounting for differences among species. (D) Species-specific arithmetic mean (± SD) equator eggshell thickness increased with species mean egg length (raw data), shown with the among-species regression line. Non-significant regression lines are represented by dashed lines.

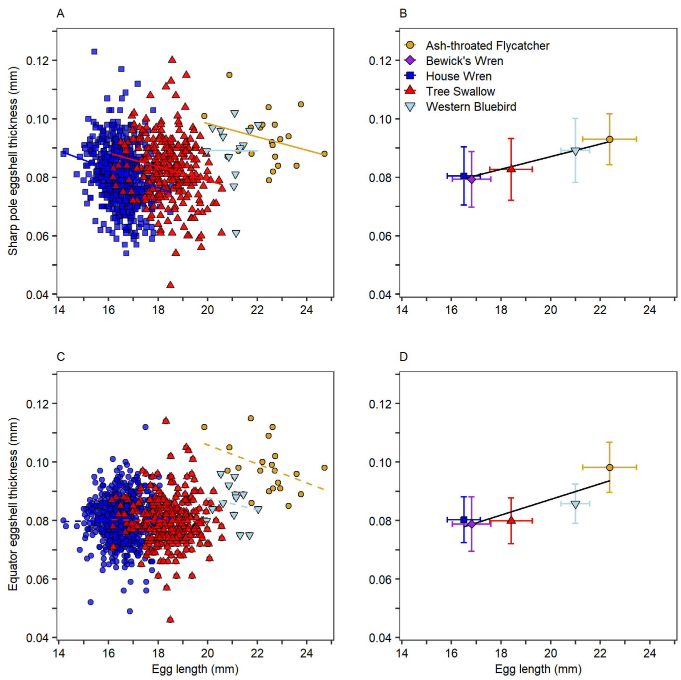
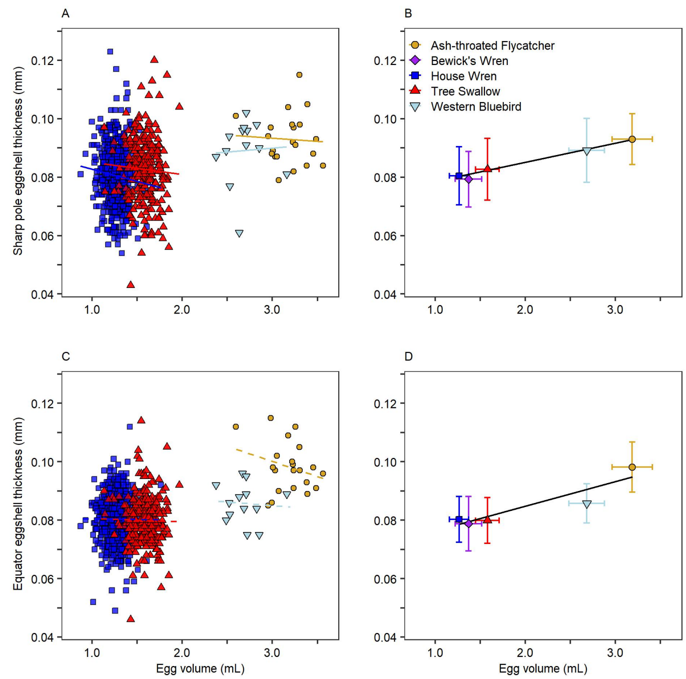


Fig. 4. Eggshell thickness at the sharp pole and at the equator as a function of egg volume within and among songbird species from California's Central Valley 2014–2018. (A) Sharp pole eggshell thickness of individual eggs decreased as a function of egg volume, after accounting for differences among species, with slopes for each species extracted from the global model. (B) Species-specific arithmetic mean (± SD) eggshell thicknesses at the sharp pole increased with species mean egg volume (raw data), shown with the among-species regression line. (C) Based on individual eggs, we did not detect a significant relationship between eggshell thickness at the equator and egg volume, after accounting for differences among species. (D) Species-specific arithmetic mean (± SD) equator eggshell thickness increased with species mean egg volume (raw data), shown with the among-species regression line. Non-significant regression lines are represented by dashed lines.



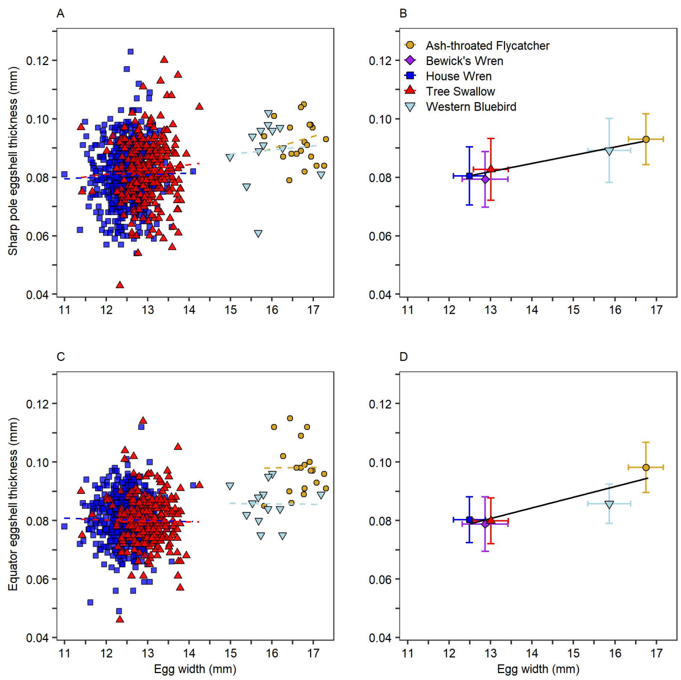
from early incubation ($n = 321$). Within Tree Swallow, late incubation eggs ($n = 6$) were 11.0% thinner at the equator ($t = 2.55$, df = 5.3, $p = 0.049$) than eggs from early incubation ($n = 181$).

Comparisons with other models

For each of the five species, we compared the mean eggshell thickness at the equator with a selection of predictive equations reported in the literature. The equations derived from Japanese Quail (*Coturnix japonica*) eggs by Khurshid et al. (2003), based on egg width, predicted eggshell thickness values to be >200% thicker than our measured means that were based on minimum eggshell thickness. Rahn and Paganelli's (1989) songbird-specific equation based on egg mass data (n = 3,929 species) from Schönwetter and Meise (1960) predicted eggshell thickness values 18% thinner, on average, than our measured eggshell thickness means (range 9–25% thinner). Ar and Rahn's (1985) regression equation based on egg mass and eggshell thickness data from 161 species (including 14 songbirds) estimated eggshell thickness values that were, on average, 10% thinner than our measured eggshell thickness means (range 19% thinner to 3% thicker). The equation that best matched our measured values was a regression equation to predict eggshell thickness from egg volume derived by Picman et al. (1996), based on ten songbird species (intercept = 0.059, slope = 0.011). This equation produced calculated values on average 4% thinner (range 9% thinner to 3% thicker among species) than our measured eggshell thickness means.

Previous work on 12 non-songbird species using the same measurement technique as the present study found a strong interspecific linear relationship between sharp pole thickness and equator thickness (Peterson et al. 2020). Using the linear predictive equation derived solely from non-songbird eggs resulted in up to an 8.0% underestimation of mean sharp pole thicknesses in the songbird species studied here (on average, 4.8% underestimation; Fig. 7*b*). A $\log_e$-$\log_e$ regression incorporating the songbird data (adjusted $R^2 > 0.99$, $F_{1,15} = 5,147.3$, $p < 0.001$; slope = 0.957, intercept = - 0.104)

Fig. 5. Eggshell thickness at the sharp pole and at the equator as a function of egg width within and among songbird species from California's Central Valley 2014–2018. (A) There was no detected relationship between sharp pole eggshell thickness of individual eggs and egg width, after accounting for differences among species. Slopes for each species were extracted from the global model. (B) Species-specific arithmetic mean (± SD) eggshell thicknesses at the sharp pole increased with species mean egg width (raw data), shown with the among-species regression line. (C) Based on individual eggs, we did not detect a significant relationship between eggshell thickness at the equator and egg width, after accounting for differences among species. (D) Species-specific arithmetic mean (± SD) equator eggshell thickness increased with species mean egg width (raw data), shown with the among-species regression line. Non-significant regression lines are represented by dashed lines.



performed better for the five songbird species studied here and only underestimated the empirical species mean sharp pole thickness measurements for the five songbird species by an average of 0.3% (range: 3.7% underestimation to 5.1% overestimation; Fig. 7*b*).

Similarly, using the linear predictive equation derived from egg length of only non-songbird eggs underestimated mean equator thicknesses by an average of 23.4% for the songbird species in the present study (range 8.3–39.4% underestimation). However, when all 16 bird species were included in the $\log_e$-$\log_e$ regression (adjusted $R^2 = 0.97$, $F_{1,15} = 474.6$, $p < 0.001$, slope = 1.169, intercept = - 5.896; Fig. 7), the predictions for songbirds improved and songbird eggshell thickness estimations were, on average, 1.5% thicker than the empirical means, ranging from a 9.2% underestimate (House Wren) to a 12.6% overestimate (Western Bluebird).

Fig. 6. Species mean eggshell thickness (arithmetic mean ± SD) at the (A) sharp pole and the (B) equator as a function of $\log_e$-transformed adult female body mass among five species of songbird (Passeriformes) from California's Central Valley 2014–2018. The significant relationship for sharp pole thickness is shown with a solid line and the dashed line indicates a non-significant relationship for equator thickness.

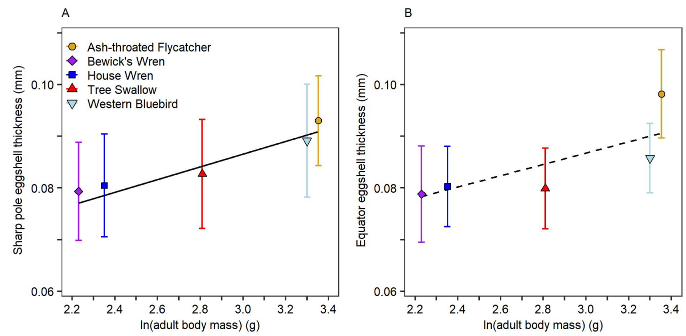
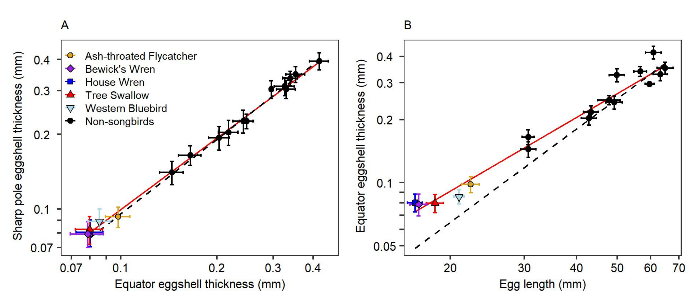


Fig. 7. (A) The relationship between species mean eggshell thicknesses (± SD; raw data) at the two measurement locations for the five songbird species analyzed from California's Central Valley 2014–2018 compared with data from the 12 non-songbird species reported by Peterson et al. (2020). We include both the linear regression line that was generated for non-songbirds only, with the line shown extended through the data from the current study (dashed black line; Peterson et al. 2020), and the $\log_e$-$\log_e$ regression line resulting from the inclusion of both songbird and non-songbird data (solid red line). (B) The relationship between species mean equator eggshell thicknesses and egg length (± SD; raw data) for the five songbird species compared with data from the 12 non-songbird species reported by Peterson et al. (2020). We include both the linear regression line that was generated for non-songbirds only, with the line shown extended through the data from the current study (dashed black line; Peterson et al. 2020), and the $\log_e$-$\log_e$ regression line resulting from the inclusion of both songbird and non-songbird data (solid red line). Note, the relationships are shown on a $\log_{10}$ axis for readability but the updated analyses that included all species were conducted using $\log_e$-transformed eggshell thicknesses and egg lengths.



DISCUSSION

Empirical eggshell thickness data for four of the species from the present study have not been reported previously and eggshell thickness for Tree Swallow eggs was reported in one prior study (Picman et al. 1996). The mean eggshell equator thickness for Tree Swallows measured in the present study (Table 1; 0.080 ± 0.008 mm) was similar to the mean reported for Tree Swallows in Ontario, Canada (0.080 ± 0.004 mm; mean ± SD; Picman et al. 1996). Although the eggshell measurements from this study contribute empirical data to the literature, eggshell thickness can vary among geographic regions, and can be affected by other environmental factors, such as contaminant exposure (Anderson and Hickey 1972, Fair and Myers 2002, Mora et al. 2007, Stein and Badyaev 2011).

Empirical data on songbird eggshell thickness are under-represented in the literature, and most studies reporting songbird eggshell thickness only include measurements at the equator (e.g., Picman et al. 1996, Mora et al. 2007, Ruuskanen et al. 2014), or average measurements across multiple or unspecified locations on the eggshell (e.g., Ar and Rahn 1985, Stein and Badyaev 2011, Bowers et al. 2015). Additionally, many studies rely on eggshell thickness indices to estimate eggshell thickness instead of obtaining empirical measurements (e.g., Fair and Myers 2002). The absence of sharp pole eggshell thickness data is likely due at least in part to the challenges inherent in measuring small eggs with traditional thickness gauges. Using a newer method (Hall-effect thickness gauge), we provide eggshell thickness measurements at both the equator and sharp pole for five species of songbirds. We found significant differences between eggshell thickness at the sharp pole and at the equator in two of the songbird species studied. Eggshells were, on average, 5.6% thicker at the equator in Ash-throated Flycatchers and 3.5% thinner at the equator in Tree Swallows. Previous work on non-songbird eggs showed that eggshells were thicker at the equator than at the sharp pole for nine of the ten waterbird species tested (Peterson et al. 2020). A study of Great Tits (*Parus major*) also found the sharp pole to be the thinnest part of the eggshell (Gosler et al. 2005). Because eggshell thickness at the poles can differ from the equator, it is important to empirically quantify eggshell thickness data for multiple egg locations.

Equator eggshell thickness is often positively related to egg length, width, volume, and adult body mass (Ar and Rahn 1985, Rahn and Paganelli 1989, Picman et al. 1996, Peterson et al. 2020), and we found that both species mean equator and sharp pole eggshell thicknesses were positively related to some egg morphometrics. Among species, those with larger eggs had thicker eggshells at the equator and the sharp pole (Figs. 4–6), which is not surprising. Moreover, species mean eggshell thickness at the sharp pole increased with $\log_e$-transformed species mean adult body mass. However, for individual songbird eggs, after accounting for species differences, we did not detect any relationships between equator eggshell thickness and egg morphometrics (e.g., length, width, volume), although sharp pole eggshell thickness was negatively related to egg length and volume (Figs. 3–4). Because equator thickness is more commonly reported in the literature, we investigated interspecific patterns using data from Peterson et al. (2020), in which they detected a strong positive relationship between species mean equator eggshell thickness and egg length among non-songbird species. The addition of the songbird data to that regression analysis did not fundamentally change the positive relationship between equator thickness and egg length (Fig. 7); however, it suggested that the overall relationship among species is non-linear. When all 16 bird species (songbirds and non-songbirds) were included in a $\log_e$-$\log_e$ regression, songbird eggshell thicknesses were estimated to be within 12.6% of empirical values.

Although our sample size for late-stage eggs was small, we detected that House Wren and Tree Swallow eggshells were thinner in the final quarter of the incubation duration than early in incubation. Embryonic development can lead to thinning of the calcite eggshell over time (Finnlund et al. 1985, Orłowski and Hałupka 2015, Orłowski et al. 2016); however, one study suggested that this thinning can be balanced by a thickening of eggshell membranes and may not be detected by methods such as the one employed here that measure the combined thickness of eggshell and membrane (Castilla et al. 2010). Additionally, eggshell thinning may not occur until relatively late in incubation and, although our songbird data set included eggs with relative embryo age ranging from 0–93% of total incubation duration, our data set was biased toward younger eggs, with 85% of measured eggs at <25% of incubation duration.

Although it is common practice to rely on predictive equations and eggshell thickness indices when empirical data are not available, our results suggest that this can result in substantial over- or underestimations of eggshell thickness, especially in smaller songbird eggs. Incorrect estimates of eggshell thickness can have important consequences for ecological or toxicological research questions. For example, errors in eggshell thickness can propagate when estimated eggshell thicknesses are used to calculate contaminant concentrations and can markedly alter calculated contaminant concentrations in egg contents (Herzog et al. 2016). Additionally, thickness indices such as the Ratcliffe Index require that the entire eggshell be weighed, and in most field studies, that is not possible. Whenever possible, it is preferable to use empirical eggshell thickness data for ecological and toxicological studies that are more specific to the species and geographic region being studied. New measurement methods allow for more precise measurements of small eggs and can preserve the integrity of eggshells (Santolo 2018).

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Data Availability:

The data that support the findings of this study are openly available in ScienceBase: https://doi.org/10.5066/P9GL21VQ

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