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Interspecific Differences in Egg Production Affect Egg Trace Element Concentrations after a Coal Fly Ash Spill

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Supporting Information

ABSTRACT: In oviparous vertebrates, trace elements transfer from mother to offspring during egg production. For animals that produce eggs slowly, like turtles, the trace element concentration of each egg reflects an integration of dietary and stored accumulation over the duration of vitellogenesis. Because turtles also produce eggs synchronously, all eggs within a clutch should exhibit uniform trace element concentrations. In contrast, for animals that produce eggs in sequence and primarily from current dietary resources, like many birds, the trace element concentrations of eggs should be less uniform within a clutch, and likely reflect short-term changes in dietary exposure. We tested the hypothesis that stinkpot turtle (*Sternotherus odoratus*) clutches exhibit lower variability and higher repeatability in barium, selenium, strontium, and thallium concentrations than those of tree swallows (*Tachycineta bicolor*) from a site impacted by a recent coal ash spill. All four trace elements exhibited significantly lower variability and significantly higher repeatability in stinkpot clutches than in swallow clutches. Mean trace element concentrations of stinkpot eggs were also significantly higher than those of swallow eggs although both species feed primarily on aquatic invertebrates. Variability in swallow egg trace element concentrations was partially due to significant laying order effects. Our results support the hypothesis that interspecific variation in the source of resources and in the synchronicity and rate of egg production can lead to interspecific differences in the variability of egg trace element concentrations.

INTRODUCTION

Maternal transfer is a significant source of exposure to bioaccumulative contaminants in oviparous vertebrates.[1−3] Maternally transferred contaminants can affect reproductive success by reducing hatching success[4−6] and by inducing developmental malformations that reduce offspring viability.[6−9] However, interspecific variation in reproductive traits like clutch size, egg size, egg production synchronicity and rate, frequency of reproduction, and resources utilized during reproduction could result in interspecific differences in maternal transfer and reproductive effects that have been relatively unexplored. Furthermore, studies of maternal transfer often examine the contaminant contents of single eggs[10−15] or pooled egg samples[16] and assume they are representative of the entire clutch. Studies demonstrating laying order effects on egg contaminant concentrations in birds suggest that this assumption is not always valid.[17−21] Within-clutch variation in egg contaminant concentrations could be the result of dietary shifts caused by heterogeneity in prey availability or nutritional needs, foraging across areas with heterogeneous contaminant "hot spots", or interspecific variability in egg production.[22,23] Because maternally transferred contaminants can strongly impact reproductive success, understanding how among-species differences in egg production arise and affect embryonic exposure is critical for assessing health risks in vertebrate assemblages exposed to bioaccumulative contaminants.

Among oviparous amniotes (birds and most reptiles), contaminants are maternally transferred during egg production, which proceeds in three stages: vitellogenesis (yolk production), albumin deposition, and eggshell formation. During vitellogenesis, phospholipoprotein yolk precursors are produced in the liver, shuttled via the bloodstream to the ovary, and deposited within ovarian follicles prior to ovulation.[24] Because yolk is a complex of protein, lipid, and inorganic nutrients,[24] vitellogenesis may be a route of maternal transfer for contaminants that incorporate into amino acids,[25] or fatty acids,[26] or replace inorganic ions.[27] In addition, yolk provides the vast majority of nutrition for embryogenesis[24] and is likely to be the primary route of maternal transfer for these contaminants.[24,25] After ovulation and fertilization, albumin and eggshell are secreted around the ova in consecutive layers by the oviduct.[28−30] Albumin is a proteinaceous complex that can be an additional source of amino acids during embryogenesis[31] and may be a route of maternal transfer of contaminants that incorporate into amino acids.[32] Eggshell can be a major source of Ca and Mg during embryogenesis in some species,[29,33] so may be a route of maternal transfer of contaminants with chemical properties similar to these ions.[27]

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Whereas the molecular mechanisms of egg production are largely conserved among oviparous amniotes,[24,34] the synchronicity and rate of egg production vary enormously, especially between birds and turtles. Furthermore, nutrients allocated to egg production may be derived from those recently assimilated from food (*income*) or mobilized from long-term storage (*capital*), the proportions of which differ among species.[32,33] Turtles provision ovarian follicles synchronously over 3−5 months[35,36] and ovulate all eggs within a clutch together.[37] Because turtles often feed throughout vitellogenesis but also maintain large fat stores,[38] they likely provision eggs with nutrients derived from income and capital sources.[39] After ovulation, albumin and eggshell are deposited on all eggs simultaneously in turtles, usually over days or weeks.[40,41] Utilization of income and capital resources, in conjunction with a very long egg production cycle, suggests that egg contaminant concentrations should reflect a long-term integration of maternal dietary exposure to contaminants. In contrast, birds establish a follicular hierarchy during vitellogenesis; yolk is deposited in follicles in a rapid and sequentially overlapping manner and ova are ovulated sequentially as they mature, usually within days of recruitment.[42−45] In addition, many passerine birds (but not all)[46,47] primarily provision income resources, and little capital, to eggs.[48,49] Birds deposit albumin and eggshell on each egg individually and oviposit one egg at a time, usually within 24 h of ovulation.[42,43] Thus, avian egg production should represent short-term contaminant exposure from primarily income resources, and to a lesser extent capital resources.[44]

The differences between birds and turtles in egg production synchronicity, rate, and resources provisioned should result in differences in maternal transfer of contaminants. In birds, diet heterogeneity, coupled with rapid and asynchronous follicular development, should result in variable contaminant concentrations within clutches. In contrast, the synchronous and extended pattern of egg production used by turtles means each egg should exhibit similar contaminant concentrations. Taken together, these considerations led us to hypothesize that in turtle and bird species foraging in the same area with overlapping dietary compositions, egg contaminant concentrations should be significantly more variable in bird clutches than in turtle clutches. We tested this hypothesis by comparing within-clutch variability of contaminant concentrations between tree swallows (*Tachycineta bicolor*) and stinkpot turtles (*Sternotherus odoratus*) inhabiting an area contaminated by trace elements from a recently remediated coal fly ash spill.

■ METHODS

Field Site Description. We examined variability in trace element concentrations in tree swallow and stinkpot eggs at the site of the Tennessee Valley Authority's Fossil Plant, Kingston, TN, U.S.A. coal fly ash spill. In December, 2008, 4.12 million cubic meters of coal fly ash were accidentally discharged into the Emory River,[50] and were swept downstream to the Clinch and Tennessee Rivers. Remediation efforts removed most ash by May 2010 (~1 year prior to our study) but 400 000 m^3 of ash remain in the system.[51] Fly-ash contains elevated concentrations of many trace elements, including arsenic (As), barium (Ba), cadmium (Cd), selenium (Se), strontium (Sr), thallium (Tl), and vanadium (V).[52] We colocated our collection efforts for both species in the Emory River within 4 km of the spill to reduce effects of spatial variability in exposure. All collections occurred in spring, 2011, about 2.5 years after the spill.

Study Species. Tree swallows are one of the primary model species used to address the movement of contaminants from aquatic to terrestrial ecosystems.[53] They are secondary cavity nesters and readily use nest boxes.[54] Both sexes remain close to their nest site throughout the breeding season and typically forage within 300−500 m of their box.[50,51,55,56] They are aerial insectivores and feed primarily on emerging aquatic insects when nesting in riparian habitats,[57] which makes them susceptible to accumulating contaminants originating from aquatic food webs.

Stinkpot turtles share several traits with tree swallows that make between-species comparisons of contaminant effects feasible. Stinkpots are small aquatic turtles that forage primarily on aquatic invertebrates, including emerging insects,[58] and are thus susceptible to exposure to trace elements in aquatic food webs.[59] Stinkpots have small home ranges, are easily trapped, and are densely populated in many aquatic habitats.

Tree Swallow Egg Collection. We installed swallow nest boxes in March 2011 and collected eggs for this study in April and May. We checked nest boxes every four days for signs of clutch initiation. If a nest contained a single egg, we numbered the egg using a nontoxic marker and returned to the box daily to mark new eggs until we collected all eggs on day 6 of egg-laying. Female swallows initiating nests in late-April typically lay 5−6 eggs per clutch and begin incubating after laying the penultimate egg.[54] We transported eggs to the lab, weighed each egg to the nearest 0.01 g, and measured its length and width to the nearest 0.01 mm. Eggs were frozen at −20 °C until preparation for trace element analysis.

Stinkpot Egg Collection. From May to June 2011, we captured gravid stinkpots using baited hoop traps. We palpated female turtles to determine their reproductive state and randomly selected 11 (of 32) gravid females for this study. We weighed each female and then induced oviposition via subcutaneous injection of 20 mg/kg of oxytocin dissolved in deionized water.[60,61] We placed injected females in plastic tubs with ~2 cm of dechlorinated water,[60] and placed the tubs in a dark room at ~25 °C. We checked females for deposited eggs every two hours. When eggs were present, we gently dried and weighed them to the nearest 0.01 g. We could not determine laying order because multiple eggs were often laid between checks. Every egg from each clutch was frozen at −20 °C. We palpated females to ensure they had deposited all eggs in their clutches, and then released them at the site of capture the day after completing oviposition.

Egg Preparation. While frozen, we removed eggshells from all eggs, deposited the yolk and albumin into autoclaved plastic scintillation vials, and allowed them to thaw. We then homogenized thawed egg contents with Teflon spatulas or by vortexing with Teflon beads. We lyophilized egg contents to a constant mass, and stored them in a desiccator. Moisture content of stinkpot eggs averaged 72.39 ± 0.01%, while moisture content of swallow eggs averaged 77.6 ± 0.01%. We transferred at least 50 mg into microcentrifuge tubes, and stored subsamples at −20 °C prior to shipment for trace elements analysis.

Trace Elements Analysis. Preliminary analyses showed that only Ba, Se, Sr, and Tl concentrations were significantly elevated in stinkpot and swallow eggs at the spill site compared to reference locations,[62,63] so we did not consider other elements. Trace element concentrations were quantified at the

Trace Element Analysis Core at Dartmouth College. Each egg sample was weighed in VWR trace metal clean polypropylene centrifuge tubes and 0.5 mL of 9:1 HNO_3/HCl (Optima grade, Fisher Scientific) was added. Individual egg subsample masses were variable but approximated 0.03 g for both species, and for standard reference materials. Egg samples were prepared for acid digestion in batches of 100 samples along with five each of blank, certified reference material, and fortified blank quality control samples. We also digested and analyzed matrix duplicates and matrix duplicate spikes at a frequency of one each per 20 samples. All tubes were lightly capped and placed into a CEM MARS Express (Mathews, NC) microwave digestion unit for an open vessel digestion. A fiber optic temperature probe was placed into a sample tube to provide temperature feedback to the MARS unit and the samples were heated to 95 °C for 45 min. The samples were allowed to cool, and 0.1 mL of H_2O_2 (Optima grade, Fisher Scientific) was added, and the microwave digestion was repeated. The samples were then diluted to 10 mL with 18 MΩ deionized water. All measurements were recorded gravimetrically.

Digested samples were analyzed for Ba, Se, Sr, and Tl by collision cell Inductively Coupled Plasma-Mass Spectrometry ICP-MS (7700x, Agilent, Santa Clara, CA). Concentrations of Ba, Se, Sr, and Tl, were measured in He collision mode (4.5 mL/min) and Se (78) was also measured in hydrogen mode (2.5 mL/min). Analytical procedures followed the general protocols outlined in EPA 6020A; the instrument was calibrated with NIST-traceable standards (DORM-2) and confirmed with NIST Oyster Tissue. Quality control measures included running one empty, metal-free falcon tube as a blank for every twenty samples. All of the samples used in this study were above detection limits (mean recoveries reported in Supporting Information Table S1).

Statistical Analysis. We compared egg concentrations of Ba, Se, Sr, and Tl between stinkpots and swallows using MANOVA, with maternal ID as a random effect to account for clutch effects. We compared egg trace element concentration variability directly by calculating within-clutch unbiased coefficients of variation (COV)[64] for each trace element in each clutch making clutch the experimental unit in our analysis. Following Sokal and Braumann,[65] we log-transformed each COV to normalize the data, and compared mean log-COV of each trace element between stinkpots and tree swallows using a MANOVA.

We also calculated repeatability (R) for each trace element in stinkpot and swallow clutches. Repeatability has recently been used to examine changes in variability of blood trace element concentrations in arctic birds.[66] Therefore, R should indicate whether the trace element concentration of a single egg is representative of the concentrations of all eggs within a clutch. Repeatability can be expressed as the intraclass correlation coefficient (ICC):[64]

$$R = \frac{S_a^2}{S^2 + S_a^2}$$

where S_a^2 is the among-groups variance component and S^2 is the within-groups variance component. We followed Hendricks[67] in deriving species-specific R values for each trace element from one-way random-effects ANOVAs run in SAS 9.3 (SAS Corporation, Cary, NC). We then calculated standard errors for R following Becker[68] in Excel (Microsoft, Redmond, WA). We used one-tailed F-tests to determine whether R was significantly greater than zero for each trace element within both species, and we compared trace element R values between species using two-tailed F-tests calculated following McGraw and Wong.[69]

Because swallow eggs were collected in order of laying, we were able to determine whether laying order affected within clutch variability in trace element concentrations, as well as egg mass and volume. We used repeated-measures ANOVA and posthoc Tukey Tests to examine changes in trace element concentrations in the first five eggs laid in each tree swallow clutch. We omitted the sixth egg because we had an insufficient sample size of six egg clutches (n = 4). If the data violated the assumption of sphericity, we used Greenhouse−Geisser corrections to reduce the chances of committing Type 1 errors. We also ranked egg trace element concentrations from lowest concentration to highest for visual comparison.

In all statistical tests, we assessed univariate normality and homoscedasticity of variance using Levene's Tests, Shapiro-Wilk Tests, and normal probability plots. Sample sizes were not large enough to test for violations of multivariate normality, so we used Pillai's Trace as a test statistic in all multivariate analyses because it is the most robust to violations of multivariate normality.[70] We followed MANOVAs with posthoc univariate ANOVAs to examine within-element differences between species. We judged two-way statistical tests at $\alpha = 0.05$ and one-way statistical tests at $\alpha = 0.025$, and presented means ±1 SE.

■ RESULTS

Stinkpot clutch sizes averaged 3.36 ± 0.31 (range 2−5 eggs), while swallow clutch sizes averaged 5.42 ± 0.19 (range 4−6 eggs). Total clutch mass in stinkpots averaged 15.62 ± 1.52 g (range 9.56−24.33 g), while swallow total clutch mass averaged 9.88 ± 0.34 g (range 8.20−11.29 g). Mean egg trace element concentrations were significantly higher in stinkpots than in tree swallows (Figure 1; Pillai's Trace = 3.543, $F_{88,316} = 27.82$, $p < 0.001$; Posthoc univariate ANOVAs Ba $F_{22,79} = 70.39$, $p < 0.001$; Se $F_{22,79} = 34.57$, $p < 0.001$; Sr $F_{22,79} = 70.26$, $p < 0.001$; Tl $F_{22,79} = 419.38$, $p < 0.001$). Mean clutch coefficients of variation (COV) also differed significantly between species (Figure 2; Pillai's Trace = 0.922, $F_{4,18} = 53.38$, $p < 0.001$), and posthoc ANOVAs showed that the COV of all four elements were significantly higher in swallow clutches than in stinkpot

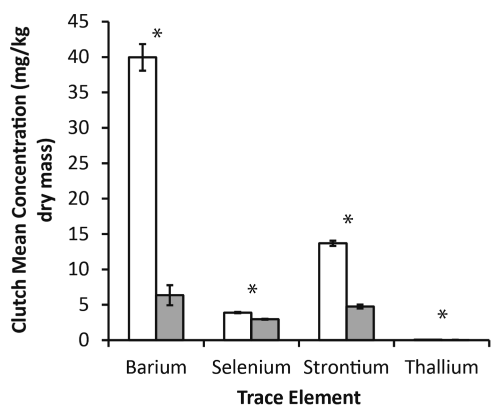

Figure 1. Mean trace element concentrations (mg/kg, dry mass) in stinkpot (white bars) and tree swallow (gray bars) egg contents from females colocated near a remediated coal fly ash spill in Kingston, TN, U.S.A. Asterisks indicate significant differences between species.

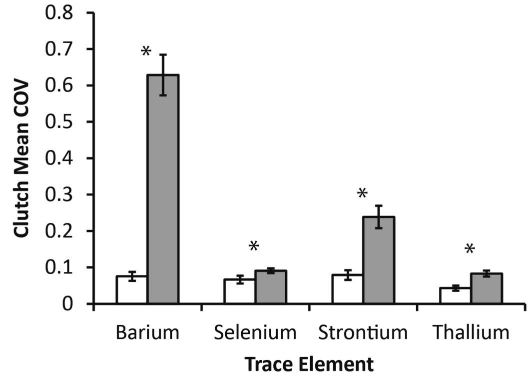

Figure 2. Mean coefficients of variation (COV) of clutch trace element concentrations in stinkpot (white bars) and tree swallow (gray bars) eggs. Asterisks indicate significant differences between species.

Table 1. *F*-Statistics and *P*-Values from One-Tailed Tests of the Hypothesis That Repeatability (*R*) Was Significantly Greater than Zero for All Four Trace Elements in Both Species ($\alpha = 0.025$)[a]

element	*F*	df	*P*
Stinkpot			
Ba	61.8*	10	<0.001
Se	39.4*	10	<0.001
Sr	19.72*	10	<0.001
Tl	318.57*	10	<0.001
Tree Swallow			
Ba	2.16	11	0.031
Se	7.03*	11	<0.001
Sr	9.42*	11	<0.001
Tl	167.31*	11	<0.001

[a]Asterisks (*) indicate whether the associated *R*-values (Fig. 3) were significantly greater than zero.

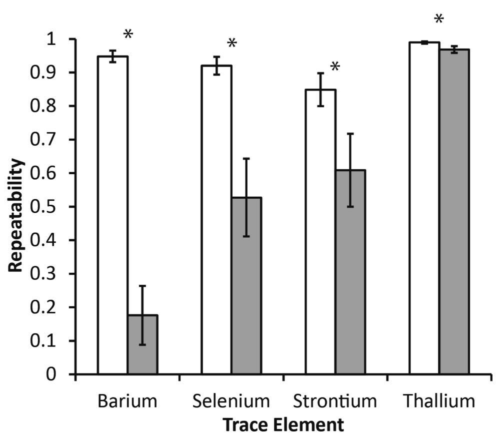

Figure 3. Repeatabilities (*R*) of clutch trace element concentrations in stinkpot (white bars) and tree swallows (gray bars) eggs. Asterisks indicate significant differences between species.

clutches (Ba $F_{1,22} = 196.59$, $p < 0.001$; Se $F_{1,22} = 6.48$, $p = 0.019$; Sr $F_{1,22} = 18.22$, $p < 0.001$; Tl $F_{1,22} = 14.68$, $p = 0.001$). Within-clutch repeatabilities (*R*) were significantly greater than zero in all species-element combinations except Ba in tree swallows (Table 1). Within-clutch R of all four elements were significantly higher in stinkpots than in swallows (Figure 3; Ba $F_{10,11} = 43.29$, $p < 0.001$; Se $F_{10,11} = 12.20$, $p < 0.001$; Sr $F_{10,11} = 4.80$, $p = 0.008$; Tl $F_{10,11} = 5.10$, $p = 0.006$).

Mean Ba, Se, and Sr concentrations changed with laying order in tree swallow eggs (Figure 4). Barium and Sr concentrations increased with laying order (Ba $F_{2,13} = 65.281$, $p < 0.001$; Sr $F_{2,12} = 13.478$, $p = 0.001$). In contrast, Se concentrations were significantly higher early in the laying order ($F_{4,32} = 19.275$, $p < 0.001$) while Tl concentrations did not change with laying order (Figure 4, $F_{4,32} = 2.301$, $p = 0.08$). Laying order effects on mean ranked concentrations were similar to those on raw concentrations. Barium and Sr concentration rank increased consecutively, while Se concentration rank decreased from egg 1 to egg 4, and Tl concentration rank did not change among eggs (Figure 4). Egg mass increased significantly with laying order ($F_{4,32} = 13.478$, $p = 0.001$) and the first two eggs laid were significantly lighter than eggs 4 and 5 (Figure 5A; all $p \leq 0.004$). We detected a similar pattern for egg volume ($F_{2,14} = 8.846$, $p = 0.005$) though the first two eggs in the clutch were only significantly lower in volume than the fifth egg (Figure 5B; both $p \leq 0.004$).

DISCUSSION

Our study demonstrated that trace element concentrations are significantly more variable within clutches of tree swallow eggs than in stinkpot eggs collected from the same site near a recently remediated coal fly ash spill, and that the variability differs among elements measured. We found that contaminant concentrations were significantly higher in stinkpot eggs than in swallow eggs, and that laying order effects differed among trace elements in swallows. The concentrations of Ba, Se, Sr, and Tl in both stinkpot and swallow eggs were below known toxic thresholds (data from birds)[23,71] and are unlikely to have severe developmental consequences. However, mean concentrations of Ba, Se, and Sr, but not Tl, were higher in the eggs of swallows and stinkpots than in some tissues of fish collected from the same location.[72] Our results highlight how differences in egg production synchronicity and rate, as well as dietary heterogeneity during egg production, may result in among-species differences in maternal transfer.

Mechanisms of maternal transfer should be conserved in stinkpots and swallows. Both species use homologous physiological mechanisms of egg production, although reptiles and birds may differ in relative proportion of specific fatty acids allocated to yolk.[73] However, mechanisms of maternal transfer likely differ among the trace elements; thus there was no a priori reason to expect that different trace elements should exhibit similar patterns of concentration variability. Barium and Sr can both substitute for Ca, and are likely to be incorporated into yolk precursors instead of Ca during vitellogenesis.[33,74] Selenium can substitute for S in cysteine and methionine,[75,76] both of which have been isolated from yolk and albumin of chicken eggs[31,77,78] and this is likely how Se enters stinkpot and tree swallow eggs. Thallium has not been studied as extensively as other trace elements, but is known to bind to sulfhydryl groups of cysteine[79] and thus may be transferred to eggs in yolk and albumin. Thallium ions also easily cross biological membranes[9] and might be passively absorbed into eggs.

Egg Variability. Barium, Se, Sr, and Tl were significantly more variable and less repeatable in tree swallow eggs than in stinkpot eggs. Despite species differences, repeatabilities of all elements except Ba in swallow eggs were significantly greater than zero. Taken together, these results are consistent with our hypothesis that sequential egg production in tree swallows results in greater within-clutch variation for these elements than

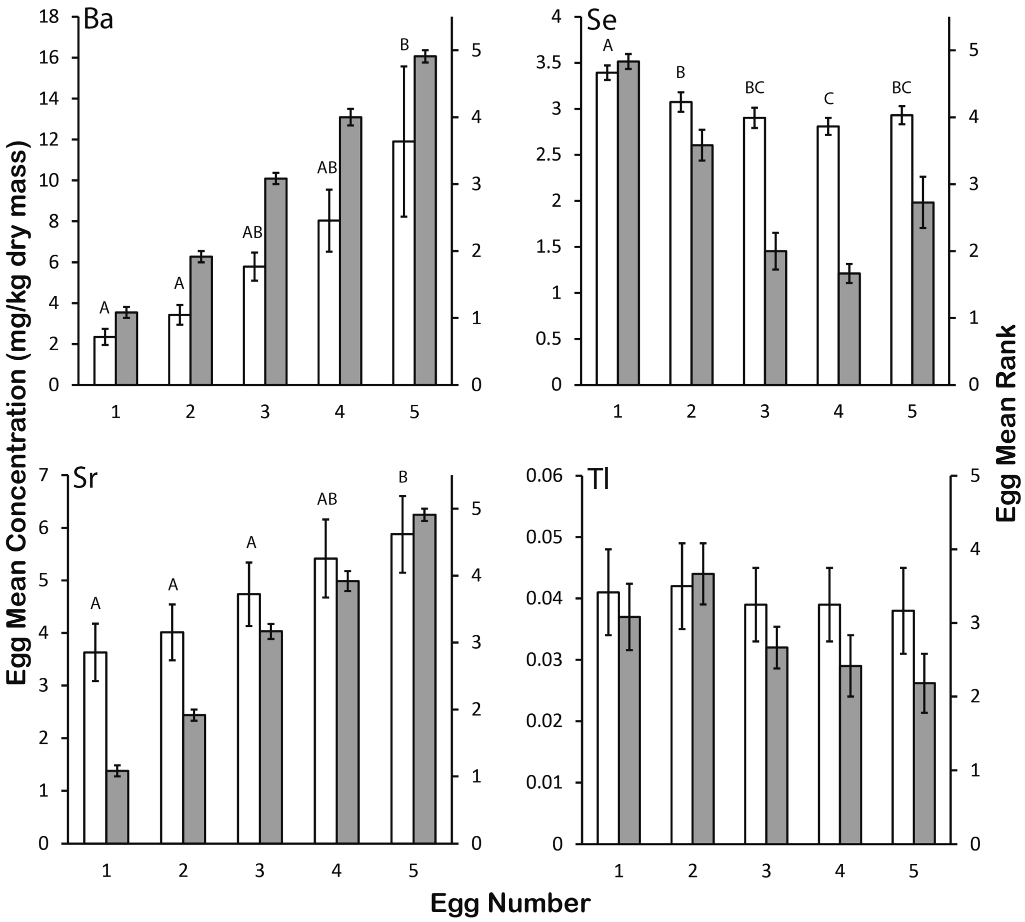

Figure 4. Egg mean concentrations (white bars, left *y*-axes) and mean ranks of concentrations (gray bars, right *y*-axes) of Ba, Se, Sr, and Tl in tree swallow eggs. Eggs are ordered from first laid (1) to last laid (5). Letters indicate significant differences among eggs. Ranks were not statistically compared.

synchronous egg production in stinkpots. In addition to sequential egg production, variability in egg trace element concentrations in swallows was likely enhanced by greater heterogeneity in their diet. As birds, tree swallows are more mobile than stinkpots, and are more likely to forage on contaminated and uncontaminated prey during egg production. Diet heterogeneity likely causes the relative amounts of trace elements maternally transferred to eggs to vary on a daily basis.[76] In contrast, stinkpots produce all eggs in a clutch synchronously,[35,36] so short-term changes in diet should affect the trace element concentrations of all eggs similarly.

Laying Order. In tree swallows, within-clutch variability was related to significant laying order effects in Ba, Se, and Sr concentrations, but not in Tl concentrations. Barium and Sr concentrations increased with laying order, while Se decreased with laying order. The coincident increase of Ba and Sr with laying order may be because both elements can replace Ca during vitellogenesis.[33,74] In other species, Se is incorporated into proteins in both yolk and albumin[17,80,81] while minerals like Ca, and hence Ba and Sr, are incorporated into yolk. This could contribute to the laying order effect we detected if the proportions of yolk and albumin change with laying order, which has been suggested for tree swallows.[44] Thallium concentrations were low in all swallow eggs and this may have inhibited our ability to detect a laying order effect if one existed. Other studies have suggested that laying order effects were more difficult to detect at low contaminant concentrations[21,82] and studies on waterbirds have reported more pronounced laying order effects at higher Se[23] and Hg[19] concentrations. Other studies that have examined the effects of laying order on the deposition of Se and other trace elements in avian eggs have found that trace elements may increase,[53] decrease,[53,83] or not change with laying order.[20,82,84−87] The inconsistency of laying order effects among studies may be due to differential availability of contaminants during egg production,[84,88,89] differences among contaminants in how they are incorporated into maternal tissue and eggs,[90] or interactions among co-occurring contaminants.[85,91] Other studies indicated that small clutch sizes seem more likely to produce a laying order effect than large clutches.[21,53] When and if stored resources are utilized in egg production may also affect contaminant deposition and produce laying order effects.[90] Methodological differences among studies may have contributed as well, because some of these studies inferred laying order or only sampled a portion of the clutch.[86,88] Many of the differences we detected occurred only between the first eggs and the last eggs laid and would have been missed had we examined only a portion of the clutch.

Egg mass and volume increased with laying order in our population of tree swallows as has been found in other populations,[44,92] but see Whittingham et al.[93] One of these studies found that later-laid eggs contained larger yolks, though changes in albumin with laying order were not addressed.[44] An increase in yolk mass could contribute to the observed increase

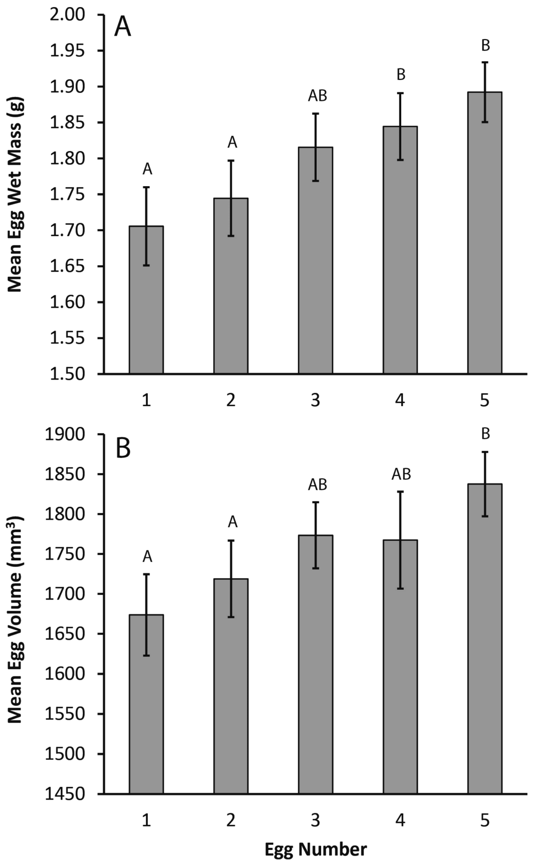

Figure 5. Egg mean wet mass (A) and volume (B) in tree swallow eggs. Eggs are ordered from first laid (1) to last laid (5). Letters indicate significant differences among eggs.

in Ba and Sr concentrations with laying order if concentrations of minerals increased concomitantly with yolk size. While Se is incorporated into yolk proteins, it is also found in albumin.[17,80,81] If increased yolk size is associated with a reduction in the amount of albumin in the egg, that could lead to the observed reduction in Se with laying order that we detected.

Mean Clutch Concentrations. In addition to differences in clutch variability, we found that Ba, Se, Sr, and Tl concentrations were all significantly higher in stinkpot eggs than in tree swallow eggs. Because vitellogenesis in particular, and egg production in general, occurs over multiple months in turtles,[35,36] it is possible that turtles maternally transfer more contaminants to their eggs simply because they consume and assimilate more contaminants during egg production than do swallows. Most of the swallow clutches used in this study were obtained from a colony where ~35% of their diet consists of terrestrial uncontaminated prey,[94] which further suggests that swallows may not consume as many contaminants during egg production as do stinkpots. Furthermore, as small songbirds, tree swallows likely do not rely heavily on capital resources for reproduction,[44,84] and should maternally transfer fewer previously bioaccumulated contaminants than do turtles which rely heavily on capital resources during vitellogenesis. For migratory passerines like tree swallows, this pattern of egg formation likely limits the sources of contaminants in their eggs to local origin. In contrast, turtles likely transfer previously bioaccumulated contaminants from long-term storage in addition to contaminants recently assimilated from diet.

In summary, our study suggests that in birds sequential egg production and greater use of heterogeneous income resources results in greater within-clutch variation in contaminant concentrations than does synchronous egg production and consumption of more homogeneously contaminated prey in turtles. In turtles, this may also contribute to higher mean egg contaminant concentrations than those found in birds. From a purely practical perspective, our results indicate that contaminant concentrations in a single egg are more likely to be representative of the mean contaminant concentrations of the entire clutch in turtles than in income-breeding birds. As a result, bird embryos may experience variable exposure to contaminants within a clutch, while turtle embryos will experience relatively similar exposure within a clutch. These differences may be especially pronounced in systems where turtles' entire home ranges are contaminated and they can forage only on contaminated prey, while birds can forage on both contaminated and uncontaminated prey. Furthermore, our results suggest that contaminant effects on reproduction in oviparous vertebrates may not be generalizable among species that differ in reproductive phenology. Further experimentation is needed to determine whether the species differences in maternal transfer we observed result in differential risks of adverse effects to developing embryos.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table S1 summarizes percent recovery and percent differences between duplicates from reference materials and blanks used for quality control during trace element analysis. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Brasso, R. L.; Abdel Latif, M. K.; Cristol, D. A. Relationship between laying sequence and mercury concentration in tree swallow eggs. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1155−1159.

(2) Nagle, R. D.; Rowe, C. L.; Congdon, J. D. Accumulation and selective maternal transfer of contaminants in the turtle *Trachemys scripta* associated with coal ash deposition. *Arch. Environ. Contam. Toxicol.* **2001**, *40* (4), 531−536.

(3) Hopkins, W. A.; DuRant, S. E.; Staub, B. P.; Rowe, C. L.; Jackson, B. P. Reproduction, embryonic development, and maternal transfer of contaminants in the amphibian *Gastrophryne carolinensis*. *Environ. Health Perspect.* **2006**, *114* (5), 661−666.

(4) Ohlendorf, H. M.; Hoffman, D. J.; Saiki, M. K.; Aldrich, T. W. Embryonic mortality and abnormalities of aquatic birds: Apparent impacts of selenium from irrigation drainwater. *Sci. Total Environ.* **1986**, *52* (1−2), 49−63.

(5) Smith, G. J.; Anders, V. P. Toxic effects of boron on mallard reproduction. *Environ. Toxicol. Chem.* **1989**, *8* (10), 943−950.

(6) Domingo, J. L. Vanadium: A review of the reproductive and developmental toxicity. *Reprod. Toxicol. Rev.* **1996**, *10* (3), 175−182.

(7) Hoffman, D. J.; Heinz, G. H. Embryotoxic and teratogenic effects of selenium in the diet of mallards. *J. Toxicol. Environ. Health* **1988**, *24* (4), 477−490.

(8) Leonard, A.; Lauwerys, R. R. Carcinogenicity, teratogenicity, and metagenicity of arsenic. *Mutat. Res., Rev. Genet. Toxicol.* **1980**, *75* (1), 49−62.

(9) Peter, A. L. J.; Viraraghavan, T. Thallium: A review of public health and environmental concerns. *Environ. Int.* **2005**, *31* (4), 493−501.

(10) Guirlet, E.; Das, K.; Girondot, M. Maternal transfer of trace elements in leatherback turtles (*Dermochelys coriacea*) of French Guiana. *Aquat. Toxicol. (Amst.)* **2008**, *88* (4), 267−276.

(11) Burger, J.; Gibbons, J. W. Trace elements in egg contents and egg shells of slider turtles (*Trachemys scripta*) from the Savannah River Site. *Arch. Environ. Contam. Toxicol.* **1998**, *34* (4), 382−386.

(12) Nyholm, N. E. I. Influence of heavy metal exposure during different phases of the ontogeny on the development of Pied Flycatchers, *Ficedula hypoleuca*, in natural populations. *Arch. Environ. Contam. Toxicol.* **1998**, *35* (4), 632−637.

(13) Burger, J.; Gochfeld, M. Heavy metal and selenium concentrations in eggs of herring gulls (*Larus argentatus*): temporal differences from 1989 to 1994. *Arch. Environ. Contam. Toxicol.* **1995**, *29* (2), 192−197.

(14) Fernie, K. J.; Bortolotti, G. R.; Smits, J. E.; Wilson, J.; Drouillard, K. G.; Bird, D. M. Changes in egg composition of American Kestrels to dietary polychlorinated biphenyls. *J. Toxicol. Environ. Health Part A* **2000**, *60* (4), 291−303.

(15) Mora, M. A. Organochlorines and trace elements in four colonial waterbird species nesting in the lower Laguna Madre, Texas. *Arch. Environ. Contam. Toxicol.* **1996**, *31* (4), 533−537.

(16) Bishop, C. A.; Brown, G. P.; Brooks, R. J.; Lean, D. R. S.; Carey, J. H. Organochlorine contaminant concentrations in eggs and their relationship to body size, and clutch characteristics of the female Common Snapping Turtle (*Chelydra serpentina serpentina*) in Lake Ontario, Canada. *Arch. Environ. Contam. Toxicol.* **1994**, *27* (1), 82−87.

(17) Heinz, G. H.; Hoffman, D. J.; Krynitsky, A. J.; Weller, D. M. G. Reproduction in mallards fed selenium. *Environ. Toxicol. Chem.* **1987**, *6* (6), 423−433.

(18) Heinz, G. H.; Hoffman, D. J.; Gold, L. G. Impaired reproduction of mallards fed an organic form of selenium. *J. Wildl. Manag.* **1989**, *53* (2), 418−428.

(19) Kennamer, R. A.; Stout, J. R.; Jackson, B. P.; Colwell, S. V.; Brisbin, I. L.; Burger, J. Mercury patterns in wood duck eggs from a contaminated reservoir in South Carolina, USA. *Environ. Toxicol. Chem.* **2005**, *24* (7), 1793−1800.

(20) Sanpera, C.; Morera, M.; Crespo, S.; Ruiz, X.; Jover, L. Trace Elements in Clutches of Yellow-Legged Gulls, *Larus cachinnans*, from the Medes Islands, Spain. *Bull. Environ. Contam. Toxicol.* **1997**, *59* (5), 757−762.

(21) Evers, D. C.; Taylor, K. M.; Major, A.; Taylor, R. J.; Poppenga, R. H.; Scheuhammer, A. M. Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* **2003**, *12* (1−4), 69−81.

(22) Colwell, S. V.; Kennamer, R. A.; Brisbin, I. L., Jr. Radiocesium patterns in Wood Duck eggs and nesting females in a contaminated reservoir. *J. Wildl. Manag.* **1996**, *60* (1), 186−194.

(23) Janz, D. M.; DeForest, D. K.; Brooks, M. L.; Chapman, P. M.; Gilron, G.; Hoff, D.; Hopkins, W. A.; McIntyre, D. O.; Mebane, C. A.; Palace, V. P.; Skorupa, J. P.; Wayland, M. Selenium toxicity to aquatic organisms. In *Ecological Assessment of Selenium in the Aquatic Environment*; Chapman, P. M., Adams, W. J., Brooks, M. L., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S., Shaw, D. P., Eds.; CRC Press: Boca Raton, FL, U.S.A., 2010; pp 141−231.

(24) Wallace, R. A., Vitellogenesis and oocyte growth in non-mammalian vertebrates. In *Developmental Biology, A Comprehensive Synthesis*; Browder, L. W., Ed.; Plenum Press: New York, NY, 1985; Vol. *1*, pp 127−177.

(25) Unrine, J. M.; Jackson, B. P.; Hopkins, W. A.; Romanek, C. S. Isolation and partial characterization of proteins involved in maternal transfer of selenium in the western fence lizard (*Sceloporus occidentalis*). *Environ. Toxicol. Chem.* **2006**, *25* (7), 1864−1867.

(26) Rauschenberger, R. H.; Sepulveda, M. S.; Wiebe, J. J.; Szabo, N. J.; Gross, T. S. Predicting maternal body burdens of organochlorine pesticides from eggs and evidence of maternal transfer in *Alligator mississippiensis*. *Environ. Toxicol. Chem.* **2009**, *23* (12), 2906−2915.

(27) Mora, M. A. Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona. *Environ. Pollut.* **2003**, *125* (3), 393−400.

(28) Stevens, L. Egg white proteins. *Comp. Biochem. Physiol. B.* **1991**, *100* (1), 1−9.

(29) Packard, M. J.; DeMarco, V. G., Eggshell structure and formation in eggs of oviparous reptiles. In *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*, Deeming, D. C.; Ferguson, M. W. J., Eds. Cambridge University Press: Cambridge, U.K., 1991; pp 53−69.

(30) Board, R. G.; Sparks, N. H. C., Shell structure and formation in avian eggs. In *Egg Incubation: Its Effects on Embryonic Development in Birds and Reptiles*; Deeming, D. C., Ferguson, M. W. J., Eds.; Cambridge University Press: Cambridge, U.K., 1991; pp 71−86.

(31) Bergmann, M.; Niemann, C. On the structure of proteins: Cattle hemoglobin, egg albumin, cattle fibrin, and gelatin. *J. Biol. Chem.* **1937**, *118*, 301−314.

(32) Richards, M. P.; Steel, N. C. Trace element metabolism in the developing avian embryo: a review. *J. Exp. Zool.* **1987**, No. Supplement 1, 39−51.

(33) Simkiss, K. Calcium metabolism and avian reproduction. *Biol. Rev. (Camb.)* **1961**, *36* (3), 321−367.

(34) Wiley, H. S.; Wallace, R. A. The structure of vitellogenin. *J. Biol. Chem.* **1981**, *256* (16), 8626−8634.

(35) Mendonça, M. T. Photothermal effects on the ovarian cycle of the musk turtle, *Sternotherus odoratus*. *Herpetologica* **1987**, *43* (1), 82−90.

(36) McPherson, R. J.; Boots, L. R.; MacGregor, R., III; Marion, K. R. Plasma steroids associated with seasonal reproductive changes in a multiclutched freshwater turtle *Sternotherus odoratus*. *Gen. Comp. Endocrinol.* **1982**, *48* (4), 440−451.

(37) Ewert, M. A. Embryology of turtles. In *Bioliogy of Reptila*, Gans, C., Billet, F., Maderson, P. F. A., Eds.; John Wiley and Sons: New York, NY, 1985; Vol. *14*, pp 76−267.

(38) Ho, S. M.; Kleis, S.; McPherson, R.; Heisermann, G. J.; Callard, I. P. Regulation of vitellogenesis in reptiles. *Herpetologica* **1982**, *38* (1), 40−50.

(39) Van Dyke, J. U.; Beaupre, S. J.; Kreider, D. L. Snakes allocate amino acids acquired during vitellogenesis to offspring: Are capital and income breeding consequences of variable foraging success? *Biol. J. Linn. Soc.* **2012**, *106* (2), 390−404.

(40) Tinkle, D. W.; Gibbons, J. W. *the Distribution and Evolution of Viviparity in Reptiles*; Museum of Zoology, University of Michigan: Ann Arbor, MI, 1977.

(41) Powell, C. B. Female sexual cycles of *Chrysemys picta* and *Clemmys insculpta* in Nova Scotia. *Can. Field-Nat.* **1967**, *81*, 134−140.

(42) Ojanen, M. Egg development and the related nutrient reserve depletion in the pied flycatcher, *Ficedula hypoleuca*. *Ann. Zool. Fenn.* **1983**, *20*, 293−300.
(43) Etches, R. J.; Petitte, J. N. Reptilian and avian follicular hierarchies: Models for the study of ovarian development. *J. Exp. Zool.* **1990**, No. Supplement 4, 112−122.
(44) Ardia, D. R.; Wasson, M. F.; Winkler, D. W. Individual quality and feed availability determine yolk and egg mass and egg composition in tree swallows *Tachycineta bicolor*. *J. Avian Biol.* **2006**, *37* (3), 252−259.
(45) Thomas, V. G., Body condition, ovarian hierarchies, and their relation to egg formation in Anseriform and Galliform species. In *Acta XIX Congresses Internationalis Ornithologici*; Quellet, H., Ed.; National Museum of Natural Science: Ottawa, ON, Canada, 1988; pp 353−363.
(46) Sénéchal, É.; Bêty, J.; Gilchrist, H. G.; Hobson, K. A.; Jamieson, S. Do purely capital layers exist among flying birds? Evidence of exogenous contribution to arctic-nesting common eider eggs. *Oecologia (Berl.)* **2011**, *165*, 593−604.
(47) Gauthier, G.; Bety, J.; Hobson, K. A. Are greater snow geese capital breeders? New evidence from a stable-isotope models. *Ecology* **2003**, *84*, 3250−3264.
(48) Ward, S.; Bryant, D. M. Barn sawllows *Hirundo rustica* form eggs mainly from current food intake. *J. Avian Biol.* **2006**, *37*, 179−189.
(49) Langin, K. M.; Norris, D. R.; Kyser, T. K.; Marra, P. P.; Ratcliffe, L. M. Capital versus income breeding in a migratory passerine bird: evidence from stable-carbon isotopes. *Can. J. Zool.* **2006**, *84*, 947−953.
(50) TVA. *Corrective Action Plan for the TVA Kingston Fossil Plant Ash Release*; Tennessee Valley Authority: Kingston, TN, 2009.
(51) Yankee, D.; McCracken, S.; Zeller, C. In *TVA Kingston Ash Recovery Status*, Proceedings of the Twenty-First Tennessee Water Resources Symposium, 2011; Tennessee Section of the American Water Resources Association: Nashville, TN, 2011.
(52) Rowe, C. L.; Hopkins, W. A.; Congdon, J. D. Ecotoxicological implications of aquatic disposal of coal combustion residues in the United States: A review. *Environ. Monit. Assess.* **2002**, *80* (3), 207−276.
(53) Custer, C. M.; Gray, B. R.; Custer, T. W. Effects of egg order on organic and inorganic element concentrations and egg characteristics in tree swallows, *Tachycineta bicolor*. *Environ. Toxicol. Chem.* **2010**, *29* (4), 909−921.
(54) Robertson, R. J.; Stutchbury, B. J.; Cohen, R. R.; Winkler, D. W.; Hallinger, K. K.; Ardia, D. R. Tree Swallow. http://bna.birds.cornell.edu/bna/species/011/articles/introduction (accessed April 10, 2013).
(55) Dunn, P. O.; Hannon, S. J. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. *Auk* **1992**, *109* (3), 488−499.
(56) Quinney, T. E.; Ankney, C. D. Prey size selection by tree swallows. *Auk* **1985**, *102*, 245−250.
(57) Custer, C. M. Swallows as sentinel species for contaminant exposure and effect studies. In *Wildlife Ecotoxicology: Forensic Approaches*; Elliot, J. E., Bishop, C. A., Morrissey, C. A., Eds.; Springer: New York, 2011; pp 45−91.
(58) Ernst, C. H.; Lovich, J. E. *Turtles of the United States and Canada*, 2nd ed.; Johns Hopkins University Press: Baltimore, MD, 2009.
(59) Bergeron, C. M.; Husak, J. F.; Unrine, J. M.; Romanek, C. S.; Hopkins, W. A. Influence of feeding ecology on blood mercury concentrations in four species of turtles. *Environ. Toxicol. Chem.* **2007**, *26* (8), 1733−1741.
(60) Ewert, M. A.; Legler, J. M. Hormonal induction of oviposition in turtles. *Herpetologica* **1978**, *34* (3), 314−318.
(61) Tucker, J. K.; Thomas, D. L.; Rose, J. Oxytocin dosage in turtles. *Chelonian Conserv. Biol.* **2007**, *6* (2), 321−324.
(62) Beck, M. L.; Hopkins, W. A.; Hawley, D. M.; Jackson, B. P. *Tree Swallow Reproductive Success and Offspring Health Following Remediation of the Kingston Fly Ash Spill*; Tennesse Valley Authority: Kingston, TN, 2013; pp 8−43.
(63) Steen, D. A.; Van Dyke, J. U.; Hopkins, W. A. *Freshwater Turtle Abundance, Morphology and Sex Ratio in Relation to a Remediated Coal Fly-Ash Spill*; Tennesse Valley Authority: Kingston, TN, 2013, pp 111−129.
(64) Sokal, R. R.; Rohlf, E. J., *Biometry: The Principles and Practices of Statistics in Biological Research*, 3rd ed.; W.H. Freeman: San Francisco, CA, 1994.
(65) Sokal, R. R.; Braumann, C. A. Significance tests for coefficients of variation and variability profiles. *Syst. Zool.* **1980**, *29* (1), 50−66.
(66) Wayland, M.; Alisauskas, R. T.; Kellett, D. K.; Mehl, K. R. Trace element concentrations in the blood of nesting King Eiders in the Canadian Arctic. *Arch. Environ. Contam. Toxicol.* **2008**, *55* (4), 683−690.
(67) Hendricks, P. Repeatability of size and shape of American Pipit eggs. *Can. J. Zool.* **1991**, *69* (10), 2624−2628.
(68) Becker, W. A. *Manual of Quantitative Genetics*; Students Book Corporation: Pullman, WA, 1975; p 170.
(69) McGraw, K. O.; Wong, S. P. Forming inferences about some intraclass correlation coefficients. *Psychol. Methods* **1996**, *1* (1), 30−46.
(70) Scheiner, S. M., MANOVA: Multiple response variables and multispecies interactions. In *Design and Analysis of Ecological Experiments*, Scheiner, S. M.; Gurevitch, J., Eds. Oxford University Press: New York, NY, 2001; pp 99−115.
(71) Ridgway, L. P.; Karnofsky, D. A. The effects of metals on the chick embryo: Toxicity and production of abnormalities in development. *Ann. N.Y. Acad. Sci.* **1952**, *55*, 203−215.
(72) Arcadis. *Trace Element Concentrations in Fish: 2010*; Tennesse Valley Authority: Kingston, TN, 2012.
(73) Speake, B. K.; Thompson, M. B. Comparative aspects of yolk lipid utilisation in birds and reptiles. *Poult. Avian Biol. Rev.* **1999**, *10* (4), 181−211.
(74) Simkiss, K. The sources of calcium for the ossification of the embryos of the giant leathery turtle. *Comp. Biochem. Physiol.* **1962**, *7* (1−2), 71−79.
(75) Unrine, J. M.; Jackson, B. P.; Hopkins, W. A. Selenomethionine biotransformation and incorporation into proteins along a simulated terrestrial food chain. *Environ. Sci. Technol.* **2007**, *41* (10), 3601−3606.
(76) Maher, W.; Roach, A.; Doblin, M.; Fan, T.; Foster, S.; Garrett, R.; Möller, G.; Oram, L.; Wallschläger, D., Environmental sources, speciation, and partitioning of selenium. In *Ecological Assessment of Selenium in the Aquatic Environment*; Chapman, P. M., Adams, W. J., Brooks, M. L., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S., Shaw, D. P., Eds.; CRC Press: Boca Raton, FL, 2010; pp 47−92.
(77) Mecham, D. K.; Olcott, H. S. Phosvitin, the principal phosphoprotein of egg yolk. *J. Am. Chem. Soc.* **1949**, *71* (11), 3670−3679.
(78) Wahli, W. Evolution and expression of vitellogenin genes. *Trends Genet.* **1988**, *4* (8), 227−232.
(79) Galvan-Arzate, S.; Santamaria, A. Thallium toxicity. *Toxicol. Lett. (Shannon)* **1998**, *99* (1), 1−13.
(80) Jacobs, K.; Shen, L. H.; Benemariya, H.; Deelstra, H. Selenium distribution in egg-white proteins. *Z. Lebensm.-Unters.-Forsch.* **1993**, *196* (3), 236−238.
(81) Latshaw, J. D.; Osman, M. Distribution of selenium in egg white and yolk after feeding natural and synthetic selenium compounds. *Poult. Sci.* **1975**, *54* (4), 1244−1252.
(82) Dauwe, T.; Janssens, E.; Bervoets, L.; Blust, R.; Eens, M. Heavy-metal concentrations in female laying great tits (*Parus major*)and their clutches. *Arch. Environ. Contam. Toxicol.* **2005**, *49* (2), 249−256.
(83) Sanpera, C.; Morera, M.; Ruiz, X.; Jover, L. Variability of mercury and selenium levels in clutches of Audouin's Gulls (*Larus audouinii*) breeding at the Chafarinas Islands, Southwest Mediterranean. *Arch. Environ. Contam. Toxicol.* **2000**, *39* (1), 119−123.
(84) Heinz, G. H., Selenium in birds. In *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*; Beyer, W. N., Heinz, G. H., Redmon-Norwood, A. W., Eds.; CRC Lewis: Boca Raton, FL, 1996; pp 447−458.
(85) Stanley, T. R., Jr.; Spann, J. W.; Smith, G. J.; Rosscoe, R. Main and interactive effects of arsenic and selenium on mallard reproduction

and duckling growth and survival. *Arch. Environ. Contam. Toxicol.* **1994**, *26* (4), 444−451.

(86) Bryan, A. L., Jr.; Hopkins, W. A.; Baionno, J. A.; Jackson, B. P. Maternal transfer of contaminants to eggs in common grackles (*Quiscalus quiscala*) nesting on coal fly ash basins. *Arch. Environ. Contam. Toxicol.* **2003**, *45* (2), 273−277.

(87) Brasso, R. L.; Cristol, D. A. Effects of mercury exposure on the reproductive success of tree swallows (*Tachycineta bicolor*). *Ecotoxicology* **2008**, *17* (2), 133−141.

(88) Longcore, J. R.; Haines, T. A.; Halteman, W. A. Mercury in tree swallow food, eggs, bodies, and feathers at Acadia National Park, Maine, and an EPA Superfund site, Ayer, Massachusetts. *Environ. Monit. Assess.* **2007**, *126* (1−3), 129−143.

(89) Morrissey, C. A.; Eilliot, J. E.; Ormerod, S. J. Diet shifts during egg laying: implications for measuring contaminants in bird eggs. *Environ. Pollut.* **2010**, *158* (2), 447−454.

(90) Nisbet, I. C. T. Eggshell characteristics and organochloride residues in common terns: Variation with egg sequence. *Colonial Waterbirds* **1982**, *5*, 139−143.

(91) Bargellini, A.; Marchesi, I.; Rizzi, L.; Cauteruccio, L.; Masironi, R.; Simioli, M.; Borella, P. Selenium interactions with essential and toxic elements in egg yolk from commercial and fortified eggs. *J. Trace Elem. Med. Biol.* **2008**, *22* (3), 234−241.

(92) Zach, R. Hatching asynchrony, egg size, growth, and fledging in tree swallows. *Auk* **1982**, *99* (4), 695−700.

(93) Whittingham, L.; Dunn, P. O.; Lifjeld, J. T. Egg mass influences nestling quality in tree swallows, but there is no differential allocation in relation to laying order or sex. *Condor* **2007**, *109* (3), 585−594.

(94) Beck, M. L.; Hopkins, W. A.; Jackson, B. P. Spatial and temporal variation in the diet of tree swallows: Implications for trace-element exposure after habitat remediation. *Arch. Environ. Contam. Toxicol.* **2013**, *65* (3), 575−587.