MERCURY PATTERNS IN WOOD DUCK EGGS FROM A CONTAMINATED RESERVOIR IN SOUTH CAROLINA, USA

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Abstract—Mercury contamination of wildlife populations has been documented widely in recent years as biomonitoring has become an important tool for assessing environmental contamination. Avian eggs provide an ideal assay material for Hg biomonitoring, particularly when the collection of eggs is simplified by using cavity-nesting species that nest in easily monitored nest boxes. However, studies are needed that address the dynamics of how Hg is distributed within eggs, and how Hg is deposited naturally within clutches laid by a single female and among clutches laid by different females occupying the same contaminated environment. We collected 138 eggs from 13 complete clutches of box-nesting wood ducks (Aix sponsa) during 1991 and 1992 at a contaminated reservoir of the U.S. Department of Energy’s Savannah River Site in South Carolina, USA. Total Hg residues in egg components and clutches were determined, partitioning of Hg among egg components was examined, and effects of egg-laying sequence on egg component Hg levels were determined. Mean albumen Hg was 0.22 ppm wet mass, mean yolk Hg was 0.04 ppm, and mean shell Hg was 0.03 ppm. On average, 86.1% of total egg Hg was concentrated in the albumen, 11.2% in the yolk, and 2.7% in the shell. Mercury concentrations in all egg components varied significantly among clutches and between successive clutches laid by the same female in the same year. Laying sequence significantly affected Hg concentrations in the albumen and shell, but not in the yolk. Declines of albumen Hg due to laying sequence were more pronounced for clutches that contained higher average Hg levels. Our results suggest that collection of first-laid eggs may be preferable for assessing maximal Hg exposure to developing embryos, and that monitoring Hg levels through the use of empty eggshells following brood departure from nests may be valid only if the laying sequence is known.

Keywords—Aix sponsa Mercury Egg-laying sequence Savannah River Site

INTRODUCTION

Mercury is a highly toxic element that has caused severe pollution problems in both terrestrial and aquatic ecosystems worldwide. Inputs of Hg to the environment largely are the result of human activities, most recently as a fungicide in agriculture, in the manufacture of chlorine and sodium hydroxide, as a slime-control agent in the pulp and paper industry, in the production of plastics and electrical apparatus, and in mining and smelting operations [1]. Local levels of Hg in soil and water are a result of natural levels, recent flooding, and local anthropogenic emissions, as well as global atmospheric transport [2]. Mercury and its compounds have no known biological function; can be bioconcentrated in organisms and biomagnified through food chains; and are mutagenic, teratogenic, and carcinogenic [3]. These characteristics give rise to concern for deleterious effects of Hg on both humans and wildlife [4]. Mercury can be present in both inorganic and organic forms (mainly methylmercury [MeHg]). Methylmercury is the most toxic form, and most exposure to birds is from MeHg, as it is accumulated preferentially in tissues of fish and other prey [5,6]. Inorganic Hg is the usual industrial form, but Hg from industrial sources may be converted into MeHg by some organisms, including birds [7–9]. Methylmercury makes up almost 100% of the total Hg in liver, kidney, muscle, and feathers of birds [10,11].

Mercury contamination of wildlife populations has been documented widely (e.g., [12–14]) as part of an increased interest in the general health of the world’s habitats and ecosystems by government agencies and the general public. Assessing ecosystem health requires diverse data on temporal and spatial trends in contamination levels, as well as effects data at population and organismal levels [15]. Biomonitoring is an important tool for assessing these trends and has been adopted at both small and large geographic scales [16,17]. Waterfowl often have been the focus of such contaminant studies because they frequently have unlimited access to wetlands in which contaminants accumulate [18] and are consumed by humans. Most studies of Hg residues in waterfowl have indicated that piscivorous ducks contained higher Hg residues than invertebrate feeders and that herbivorous ducks usually showed the lowest Hg levels [19,20]. Although invertebrate feeders and herbivorous birds have lower concentrations of Hg than waterfowl feeding at higher trophic levels, they still have been the focus of several contaminant studies [21–23]. Wood ducks (Aix sponsa) have been used in several contaminant studies [23–25] because they have a very wide geographical distribution in North America and can be studied easily because they are attracted to nest boxes.

The avian egg has been used in numerous studies to monitor
contaminants [13,14,26,27] because it has several advantages over internal tissues. Eggs have a highly consistent composition, are produced by a clearly identified segment of the population, and can be collected and handled easily; in addition, their removal places little drain on the population [16]. This study examined variation in total Hg for complete clutches of wood duck eggs from a contaminated reservoir of the Savannah River Site (SRS) in South Carolina. Although Hg contamination of waterfowl eggs has received prior attention [22,28,29], wood duck eggs have not yet been the focus of such a contaminant study because the birds are primarily herbivorous [30]. Wood ducks are ideal for study in this case, however, because they are a year-round resident of South Carolina, are the only waterfowl species that commonly nests on the SRS, lay large clutches, have been studied at length at this site, and are hunted extensively in South Carolina [31–33].

One objective of this study was to determine the levels of total Hg residues in components (albumen, yolk, and shell) of wood duck eggs. In Japanese quail (Coturnix coturnix), Hg particularly accumulated in the albumen after exposure to MeHg [34]. We also examined whether the albumen and yolk Hg concentrations could be estimated adequately from shell Hg concentrations because egg shells and their associated chorio-allantoic membranes (CAMs) frequently are the only remnants of eggs in nests following hatching and, thus, they could provide a nondestructive opportunity to assess contaminant levels [35,36]. Heinz and Hoffman [36], for example, recently found that Hg in CAMs at hatching and neonate ducklings (Anas platyrhynchos) eggs were related positively, but their sample size was small and the relationship was somewhat variable.

Another objective of this study was to examine among- and within-clutch variation in egg component Hg concentrations. We sought to identify behavioral patterns that might explain Hg deposition in clutches laid by different females and in multiple clutches laid by the same female in the same year. We examined within-clutch Hg concentrations to determine if laying sequence affected Hg levels in wood duck egg components. A relationship between egg order and Hg level was observed in the two to three egg clutches of common terns (Sterna hirundo) [37] and herring gulls (Larus argentatus) [38], and was suggested by Zicus et al. [29] following a study of 1,1-dichloroethylene bis (p-chlorophenyl) [DDE], polychlorinated biphenyls, and Hg residues in waterfowl eggs. However, others have suggested that eggs of the same clutch generally show similar Hg levels and, thus, any one egg reflects the contamination of the entire clutch and female [39,40].

Given the paucity of studies examining the effect of laying sequence on Hg contamination and the relatively small clutch size of the species examined, our study serves to investigate a question essential to the use of avian eggs as monitoring units for Hg contamination using a species that lays larger clutches. We specifically tested the hypotheses that laying sequence would have no overall (i.e., clutches pooled) effect on total Hg levels in egg components, and individual clutches would not differ from the overall no-sequence effect.

MATERIALS AND METHODS

Study site
The U. S. Department of Energy’s SRS is a 780-km² nuclear production and research facility in west central South Carolina (33.1°N, 81.3°W). Our study site, Pond B, is an 87-ha abandoned reactor cooling impoundment on the SRS. Pond B was created in 1958 by impounding the headwaters of Joyce Branch and received Hg input from the pumping of make-up water from the nearby Savannah River to replace water lost by evaporation from the reservoir and seepage at the reactor. Savannah River water was contaminated with Hg primarily from chloralkali plants that were discharging effluents into the river upstream from the SRS cooling-water intake. Through 1963, Pond B was used as part of a recirculating cooling reservoir system for a nuclear production reactor until that reactor was shut down in 1964. A more detailed description of the Pond B reservoir ecosystem, flora, and fauna is provided by Whicker et al. [41].

Egg samples
Between March and June of 1991 and 1992, we collected 138 freshly laid wood duck eggs from 25 nest boxes established around Pond B (6 clutches in 1991 and 7 clutches in 1992). These eggs initially were collected during daily nest box checks in a study by Colwell et al. [42] of radiocesium (137Cs) levels in wood duck eggs and components. All collected eggs were replaced with hard-boiled chicken eggs to prevent laying females from deserting or compensating for the taken eggs. Because wood ducks lay at the rate of one egg/d [43], the appearance of ≥two eggs/d is an indication of dump nesting [44], in which more than one female lays eggs in the same nest box. Using the above criterion when collecting eggs from nest boxes on a daily basis, we attributed each clutch used in this study to a single female. After each complete clutch of eggs had been collected, all substituted chicken eggs were removed from the nest box, thereby simulating clutch loss to encourage renesting by the female; two of the 1991 clutches were renests of females that had laid clutches earlier in that same year. Clutch sizes ranged from eight to 15 eggs ($x = 10.6$, standard error $[SE] = 0.55$). Eggs were transported to the laboratory and weighed to the nearest 0.01 g and boiled in water to facilitate the separation of the shell (including shell membrane), yolk, and albumen. Boiling or heating tissue does not change or reduce Hg [45,46]. Furthermore, laboratory experiments with herring gull egg shells indicated that there is no significant difference in Hg levels in boiled and unboiled shells ($n = 6$, J. Burger, unpublished data). Egg components were separated on the day of collection and weighed to the nearest 0.01 g, dried to a constant mass at 70°C, and reweighed to the nearest 0.01 g before being placed in 20-ml plastic vials for storage. Following the completion of the Colwell et al. [42] work, we conducted Hg analyses on these same egg component samples. It is important to note that the shell membrane that was included with the egg shell in our study is not the same membrane tissue (CAM) typically collected from nests following hatching and assayed for contaminants (see Burley and Vadhehra [47] for egg membrane descriptions).

Hg analyses
Homogenized aliquots of the dry egg components (~0.2 g) were microwave digested in HNO₃ and H₂O₂. Total Hg levels in all egg component samples initially were determined at the Soils Testing Laboratory of the University of Georgia (UGA; Athens, GA, USA), using an inductively coupled plasma-mass spectrometer (ICP-MS). However, analyses of the shell/membrane material using the UGA ICP-MS analytical methodology produced Hg values that were low and highly variable, and quality control information associated with that data could not be provided. Therefore, we regarded the shell Hg data pro-
duced by this methodology as suspect and sought an alternative method (see below) for quantifying shell Hg. Albumen and yolk samples were analyzed at UGA by ICP-MS in batches with certified reference materials (CRM), blanks, and spiked samples. Recoveries of spiked blanks and samples, using the UGA ICP-MS methodology, ranged from 82 to 126%. Mean recoveries of Hg for the CRM Tort-1, Dorm-1, and Dolt-1 (National Research Council, Ottawa, ON, Canada) were 100, 106, and 124%, respectively (n = 3 for each CRM). Methodological detection limits for albumen and yolk Hg, using the UGA ICP-MS, were 0.034 and 0.007 ppm Hg, respectively. We identified seven outlier Hg values (1 albumen and 6 yolk samples) produced by the UGA ICP-MS method for re-analysis. We also selected additional albumen and yolk samples for re-analysis that were not suspect, yielding a total of 44 samples (22 each of albumen and yolk). Twelve of 13 clutches were represented by samples selected for re-analysis. These albumen and yolk samples were re-analyzed at the Savannah River Ecology Laboratory (SREL; Aiken, SC, USA) by ICP-MS in analytical procedures that included CRM and blanks. Mean recovery of Hg for Tort-2 (National Research Council) was 126% of the mean certified value (n = 3), or 103% of the upper 95% confidence interval established for Hg in Tort-2. The methodological detection limit for Hg, using the SREL ICP-MS, was 0.019 ppm Hg. A crosscomparison of the albumen and yolk Hg concentrations produced by the UGA ICP-MS and SREL ICP-MS determined that the two analytical systems were in close agreement (Spearman Correlation: Albumen [excluding one UGA ICP-MS outlier], r_s = 0.94, n = 21, p ≤ 0.0001; Yolk [excluding six UGA ICP-MS outliers], r_s = 0.82, n = 16, p ≤ 0.0001). The SREL ICP-MS methodology produced Hg concentrations that we deemed valid for all identified outlier albumen and yolk Hg data and, thus, the SREL ICP-MS values were inserted into the data set.

Additional sample aliquots of all 138 shell samples were digested and analyzed for total Hg by cold vapor atomic fluorescence spectroscopy (CVAFS) at SREL. Because of the general difficulty associated with ICP-MS analysis of sample matrices containing high levels of dissolved salts, especially non-volatile ions such as the Ca typically found in egg shells, CVAFS was chosen as a more appropriate analytical technique for determining Hg levels in our shell samples. To maximize the Hg signal while using the CVAFS methodology to analyze shell samples, a maximum amount of sample material (0.5 g) and minimal acid volume (2.5 ml) were used in the digestion process. All samples were analyzed in batches with CRM, blanks, sample digestion duplicates, sample analysis replicates, and spiked samples. Recoveries of spiked shell samples, using the CVAFS method, ranged from 91 to 110%. Mean recovery of Hg for Tort-2 (n = 15) was 103%. The method detection limit for Hg, using the CVAFS, was 0.003 ppm Hg. All 138 shell Hg concentrations produced by the CVAFS methodology were used in subsequent statistical analyses. Dry-mass Hg concentrations of all component samples were converted to wet-mass concentrations using dry/wet ratios for the albumen (0.129), yolk (0.564), and shell (0.834) determined by Kennamer et al. [48] for these same egg components.

Data analyses

Egg component Hg concentration (ppm, wet mass) distributions were examined for normality using Kolomogorov-D statistics [49]; tests of hypotheses that these Hg values were random samples from normal distributions were rejected for albumen and shell Hg data (p > 0.01), and stem-and-leaf plots suggested a log_{10}-transformation of the data before certain analyses (see below). Yolk Hg data required no transformation before analysis. Two-tailed Wilcoxon rank-sum tests [49] were used to determine if within-clutch mean Hg concentrations (ppm, wet mass) in egg components differed between years. Regression analyses [49] were conducted to determine the relationship of each egg component’s total Hg (mg) to whole-egg total Hg (mg). For each egg, the albumen, yolk, and shell component total Hg (mg) was estimated by multiplying that egg’s particular component concentration (ppm, i.e., mg/kg) by the mass of that fresh component (kg). Whole egg total Hg (mg) then was estimated as the sum of the component Hg contents. Regression analyses also were conducted to assess the predictability of albumen and yolk Hg concentrations from shell Hg concentrations. A one-way analysis of variance [49] was used to determine if Hg concentrations in components (log_{10} transformed albumen and shell Hg concentrations) differed among nests, and was followed by Bonferroni i-tests to compare mean Hg concentrations between individual nests. For eggs one to eight (a minimum of 8 eggs existed for every clutch) of clutches with known complete laying sequences (n = 9 clutches) arithmetic means (± SE) are presented graphically. To examine statistically the relationship between component levels of Hg contamination and egg-laying order, we used homogeneity of slopes models [49] examining the simultaneous effects (i.e., we interpreted Type III [partial] sums of squares) of egg sequence as a continuous variable, a main class effect due to different nests (i.e., the intercepts effect), and an interaction effect (i.e., the among-clutch slopes effect). Because clutch size varied among the nests, and we nevertheless desired to include the data for as many eggs as possible in the egg-sequence analyses, we described the position of each egg within its respective clutch as a proportion of the completed clutch (i.e., all values were between 0 and 1), thereby controlling for the various clutch sizes. The sequence of the first two to three eggs was uncertain in four clutches, and data from those entire clutches were excluded from all egg sequence analyses. While searching for egg-laying sequence effects in the homogeneity of slopes model, we used relative Hg concentrations that were expressed as deviations from within-clutch means (e.g., relative yolk Hg_{egg, i, clutch, j} = \frac{\text{yolk Hg}_{\text{egg, i, clutch, j}} - \bar{X}_{\text{yolk Hg}_{\text{clutch, j}}}}{\text{yolk Hg}_{\text{clutch, j}}}). Stope estimates of the egg-sequence effects were produced for each egg component of each clutch using estimate statements in the models. Geometric means are presented in addition to arithmetic means when appropriate, and we considered results of all tests significant at p ≤ 0.05.

RESULTS

Mercury levels and relationships among egg components

Within-clutch mean Hg concentrations did not differ by year for the albumen (Z = 0.5, p = 0.62), yolk (Z = 0.64, p = 0.52), or shell (Z = -0.21, p = 0.83); therefore, samples were pooled with regard to year for further analyses. Albumen Hg levels averaged 0.219 ppm wet mass (n = 138, SE = 0.011, geometric mean = 0.182); yolk Hg levels averaged 0.040 ppm wet mass (n = 138, SE = 0.002), and shell Hg levels averaged 0.034 ppm wet mass (n = 138, SE = 0.002, geometric mean = 0.028). On average, 86.1% (SE = 0.43) of the total egg Hg was concentrated in the albumen, 11.2% (SE = 0.44) in the yolk, and 2.7% (SE = 0.08) in the shell. Mercury in all egg components increased with increasing whole-egg Hg (Fig. 1A).
to C), with albumen, the greatest concentrator of Hg, showing
the strongest relationship to whole-egg.

A relatively weak linear relationship was demonstrated be-
tween Hg concentrations in yolk and shell \( (r^2 = 0.25, p \leq 0.0001; \text{Fig. 2A}) \), but the relationship between albumen and
shell Hg concentrations was somewhat stronger \( (r^2 = 0.61, p \leq 0.0001; \text{Fig. 2B}) \).

**Variation in egg component Hg levels among clutches**

Comparison of Hg concentrations among clutches (Table 1) showed significant differences for all egg components (al-
bumen: \( F_{12,125} = 90.11, r^2 = 0.90, p < 0.0001 \); yolk: \( F_{12,125} = 16.27, r^2 = 0.61, p < 0.0001 \); shell: \( F_{12,125} = 15.99, r^2 = 0.61, p < 0.0001 \)). For albumen, individual clutch means ranged from 0.07 to 0.49 ppm wet mass Hg; yolk clutch means
ranged from 0.014 to 0.069 ppm wet mass Hg, and shell clutch
means ranged from 0.013 to 0.069 ppm wet mass Hg (Table 1).

In 1991, two females (94806 and 94827) laid second clut-
ches in Pond B nest boxes following the removal of their first
clutches, thus providing an opportunity to examine Hg levels
in successively laid clutches of the same year. Average Hg
concentrations in egg components of 94806’s first clutch (2391,
Table 1) were higher (significant only for albumen) than those
in her second clutch (2491, Table 1); in the other case, 94827’s
first clutch (0591, Table 1) contained lower (although none
significant) component Hg concentrations than those of her
second clutch (0491, Table 1). Clutch size, however, was great-
er in both first clutches (11 and 12 eggs in 0591 and 2391,
respectively) than in second clutches (8 and 10 eggs in 0491
and 2491, respectively). Because first clutches contained more
eggs than second clutches, total Hg deposited into both first
clutches (0.042 and 0.144 mg in 0591 and 2391, respectively)
exceeded that for their respective second clutches (0.035 and
0.077 mg in 0491 and 2491, respectively).

**Egg component Hg levels and laying sequence**

Mean Hg concentrations in the albumen steadily declined
through the first five eggs laid, ranging from 0.33 ppm in first-
laid eggs to near 0.20 ppm in eggs 6 to 8 (Fig. 3A). In the
yolk, mean Hg concentrations ranged from 0.048 ppm in first-
laid eggs to 0.037 ppm in the sixth eggs (Fig. 3B), but through-
out the sequence, Hg in yolk was highly variable when com-
Table 1. Arithmetic mean ± 1 standard error (SE) of wood duck egg component total Hg (ppm, wet mass) for individual nests and for all 138 eggs (overall) from Pond B, Savannah River Site (SC, USA)^a

<table>
<thead>
<tr>
<th>Albumen</th>
<th>Yolk</th>
<th>Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nest ID</strong></td>
<td><strong>X ± SE</strong></td>
<td><strong>Bonferroni test</strong></td>
</tr>
<tr>
<td>2391(12)</td>
<td>0.494 ± 0.016</td>
<td>A</td>
</tr>
<tr>
<td>2392(12)</td>
<td>0.352 ± 0.026</td>
<td>B</td>
</tr>
<tr>
<td>2491(10)</td>
<td>0.299 ± 0.026</td>
<td>B,C</td>
</tr>
<tr>
<td>20B92(9)</td>
<td>0.270 ± 0.030</td>
<td>B,C</td>
</tr>
<tr>
<td>0292(9)</td>
<td>0.271 ± 0.012</td>
<td>B,C</td>
</tr>
<tr>
<td>1792(12)</td>
<td>0.265 ± 0.028</td>
<td>C</td>
</tr>
<tr>
<td>1191(8)</td>
<td>0.164 ± 0.013</td>
<td>D</td>
</tr>
<tr>
<td>0491(8)</td>
<td>0.155 ± 0.010</td>
<td>D</td>
</tr>
<tr>
<td>0791(10)</td>
<td>0.141 ± 0.007</td>
<td>D</td>
</tr>
<tr>
<td>0591(11)</td>
<td>0.134 ± 0.006</td>
<td>D</td>
</tr>
<tr>
<td>2092(15)</td>
<td>0.121 ± 0.006</td>
<td>D</td>
</tr>
<tr>
<td>1592(10)</td>
<td>0.080 ± 0.002</td>
<td>E</td>
</tr>
<tr>
<td>0892(12)</td>
<td>0.070 ± 0.005</td>
<td>E</td>
</tr>
<tr>
<td>Overall</td>
<td>0.219 ± 0.011 (0.182)</td>
<td>F</td>
</tr>
</tbody>
</table>

^a Means within columns designated with the same letter in the Bonferroni tests do not differ significantly. Nests 2391 and 2491 are the first and second nests of female 94806 in 1991, respectively. Nests 0591 and 0491 are the first and second nests of female 94827 in 1991, respectively.

Overall values are component means ± SE (geometric means) of all 138 samples.

For both albumen and shell, these significant laying-order effects indicated declines in component Hg concentration in shell also was significant (egg sequence: F = 13.76, R^2 = 0.231, p < 0.0001; full model: F = 13.88, R^2 = 0.36, p = 0.0073).
examined laying sequence was not significant \(F_{17,70} = 1.48, r^2 = 0.26, \ p = 0.13\). Our hypothesis that laying sequence would have no effect on Hg levels in egg components, therefore, was rejected for the albumen and shell and accepted for the yolk. The among-clutch slope effect for albumen was significant (interaction: \(F_{8,70} = 9.77, p < 0.0001\)), indicating that differences existed among clutches for the laying sequence effect. Although all nine egg sequence slope estimates produced by the albumen model were negative, only six of the nine negative slopes were significantly different from (i.e., less than) a slope of zero. Mean clutch albumen Hg levels were related \(r_5 = -0.75\) to the magnitude of the sequence effect slopes (Fig. 4), meaning that sequence declines in albumen Hg were more pronounced for clutches that contained more sequence estimates were negative for seven of nine clutches, but only three of those clutches had negative slopes that were significantly different from (i.e., less than) zero. Neither of the two positive shell Hg slopes was significantly greater than zero. Our hypothesis that individual clutches would not differ from the overall no-sequence effect again was rejected for the albumen and shell, but was accepted for the yolk.

**Discussion**

**Relative mercury levels in egg components**

Average Hg concentrations in wood duck egg components from Pond B on the SRS were comparable to other Hg concentrations reported in primarily herbivorous waterfowl. Mercury concentrations in wood duck albumen averaged 0.22 ppm wet mass, slightly higher than 0.16 ppm reported in albumen of northern pintails \(*Anas acuta*\) in eastern North Dakota [50]. Mean Hg concentration in wood duck egg shell was 0.034 ppm wet mass, slightly lower than 0.05 ppm for mallard egg shells from Michigan [51]. Geometric mean shell Hg concentration was 0.028 ppm for wood ducks, lower than both the 0.08 ppm reported for canvasbacks \(*Aythya valisineria*\) from Nevada and the 0.04 ppm found for canvasbacks from Saskatchewan and Manitoba [28].

The majority (86.1%) of total egg Hg was found in the albumen of wood duck eggs from the SRS, as also was found for the eggs of Japanese quail that were dosed experimentally [34]. Yolk comprised 11.2% of the total Hg and shell only 2.7% of the total egg Hg for wood ducks at SRS. This differential deposition of Hg partly can be ascribed to differences in the origin of the yolk and albumen. Yolk proteins are synthesized in the liver, and albumen constituents (primarily water and protein) are synthesized in the oviduct [52] and secreted into the albumen. Albumen proteins are derived from serum proteins [52], suggesting that albumen Hg levels mainly reflect recent dietary uptake of Hg as opposed to mobilization from accumulated storage tissues. In wood ducks, yolk formation occurs over a 7-d period followed by 24 h during which albumen and shell are deposited before the egg is laid [53]. This process of egg formation, along with the differential deposition of Hg we observed, suggests that Hg levels in the albumen and shell of wood duck eggs likely represent Hg intake from the local breeding site immediately before and during egg laying, and yolk Hg levels probably reflect contamination of habitats used while accumulating nutrients that are stored in the body before reproduction. Because wood ducks on the SRS are year-round residents, Hg levels in albumen, shell, and yolk of their eggs almost certainly reflect local environmental Hg availability. These conclusions, however, should be viewed as being applicable only to wood ducks and other similar-sized precocial bird species that share similar egg-production characteristics. Further toxicodynamic examination of eggs from other species with contrasting patterns of nutrient deposition into eggs or different migratory habits will be required to further broaden our understanding of natural Hg deposition in avian eggs. Of particular interest, for example, would be the Hg contamination patterns of migratory species in which yolk-deposited Hg likely would be accumulated on southern wintering areas and albumen-deposited Hg would be accumulated upon return to northern breeding areas.

If the transfer of ingested Hg into eggs occurs as quickly in wood ducks as noted in laying chickens [54], then female wood ducks that vary their use of differentially contaminated foraging sites should show differences in egg Hg concentrations. Mercury concentrations in all components differed significantly among clutches. Such variability in Hg levels among clutches may be the result of differential Hg availability and the use of alternative wetland foraging sites in the local SRS landscape. To determine habitat use and possible foraging areas away from Pond B, three of the wood duck females captured on their nests were fitted with radio transmitters and released the same day to the same nest boxes [42]. Telemetry information collected during the laying cycle of these three females producing clutches used in this study indicated the use of at least three foraging areas away (≤1 km) from Pond B [42]. All three identified areas were ephemeral depression wetlands that were flooded in 1991 and thus were available as alternate foraging sites for wood ducks nesting at Pond B; only two of these three alternate foraging sites were available in 1992. Temporal variation in availability of alternative foraging sites resulting from fluctuating rainfall, and the preferences of individual females for certain foraging areas and food types, probably all play roles in determining Hg levels in clutches of Pond B wood ducks. Our results indicate that when using wood ducks as monitors of environmental Hg contamination, the collection of eggs from several clutches over a period of years would be necessary to capture the variance associated with differential habitat use by individual nesting females.

For both renesting females in our study, levels of Hg con-
Hg patterns in wood duck eggs

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was common for all components. Significant negative relationships between laying order and Hg concentration existed for the albumen and shell, but were not found for the yolk. For all nests, Hg in the albumen tended to decline as the laying sequence progressed, usually with the first egg of the clutch containing the highest albumen Hg concentration, as reported for Charadriiformes by Becker [38]. Notably although, laying sequence declines of albumen Hg were more prominent within clutches that contained higher average Hg contamination, thus explaining why slopes differed significantly among nests. However, no clutches in our study exhibited significant positive slopes, so Hg never increased through a laying sequence. These results for the albumen also are consistent with the notion that albumen Hg concentrations represent recent Hg intake from the breeding area during egg-laying. Relatively higher levels of Hg in the albumen of the first egg(s) may result from the accumulation of Hg immediately before nesting when females switch to a higher protein (invertebrate) diet so that much of that recent higher Hg burden is deposited immediately into the earliest laid eggs of the clutch. For yolk, mean Hg concentrations fluctuated through the egg sequence with no significant pattern. The variable nature of Hg concentrations we observed in yolk throughout the laying sequence may be expected because endogenous nutrients and their associated Hg are integrated over several days during yolk formation.

This study indicates that wood duck eggs can be used effectively as biomonitor of Hg contamination, and the significant relationship of laying sequence to Hg contamination in egg components, particularly in the albumen, the greatest Hg concentrator, is important for establishing an adequate sampling protocol. Our results suggest that identifying and collecting all first-laid eggs may be preferable, if not essential, for assessing maximal contaminant exposure to developing embryos.

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REFERENCES


tamination differed from first clutches to second clutches with no consistent patterns between females. These differences, along with the physiology of wood duck egg formation described above, suggested that the degree of foraging on the contaminated reservoir between nestings was important in determining the amount of Hg deposited in second clutches of wood ducks nesting at Pond B. Results from a study of $^{137}$Cs in these same wood duck eggs suggested a similar explanation for the observed patterns of variation of that contaminant [42].

In a study of Hg residues in wood duck foods of eastern Tennessee, USA, Lindsay and Dimmick [23] found Hg levels several times greater in invertebrate prey items compared to plant foods. Additionally, Landers et al. [30] reported that female wood ducks of the SRS increased their invertebrate intake during breeding to comprise a high of 23% of the diet in March. Such differences in invertebrate intake for female wood ducks, together with varying levels of Hg contamination and availability of food items, also may have contributed to the differences in Hg levels observed both among clutches and between first and second clutches of the same female.

Finding strong relationships in Hg content among the egg components was a desirable outcome of this study because, in the use of wood duck eggs as monitors of environmental Hg levels, the simple collection of egg shells and/or CAMs from nests following successful hatching would greatly simplify the monitoring of Hg levels in these birds [36]. By marking the eggs during laying and subsequently collecting the egg remnants soon after hatching, levels of Hg contamination in egg shells and/or CAMs could be determined and albumen Hg levels predicted with low-impact field methods that would require no destructive sampling of the population. Heinz and Hoffman [36] compared Hg levels in 12 mallard ducklings and, thus, attention to material digestion and analytical protocols. Our results suggest that identifying and collecting all first-laid eggs may be preferable, if not essential, for assessing maximal contaminant exposure to developing embryos.

Laying-sequence effects

Results from our analyses of laying-order effects on egg components showed that no single pattern of Hg contamination appeared no consistent patterns between females. These differences, along with the physiology of wood duck egg formation described above, suggested that the degree of foraging on the contaminated reservoir between nestings was important in determining the amount of Hg deposited in second clutches of wood ducks nesting at Pond B. Results from a study of $^{137}$Cs in these same wood duck eggs suggested a similar explanation for the observed patterns of variation of that contaminant [42].

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