The Condor 109:199–205

POTENTIAL ECOTOXICOLOGICAL SIGNIFICANCE OF ELEVATED CONCENTRATIONS OF STRONTIUM IN EGGSHELLS OF PASSERINE BIRDS

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Abstract. We investigated the occurrence and potential ecotoxicological significance of elevated concentrations of strontium (Sr) in eggshells of nine passerine birds from four regions in Arizona. Concentrations of Sr in eggshells ranged from 70 to 1360 $\mu g\ g^{-1}$ dry weight (overall mean = 684 ± 345 SD $\mu g\ g^{-1}$ dw) for the four regions. 23% of the eggshells had Sr concentrations greater than 1000 $\mu g\ g^{-1}$ dw. To our knowledge, these are among the highest levels of Sr that have been reported in bird eggshells in North America. Of the nine species, Brown-headed Cowbirds (*Molothrus ater*) had the greatest concentrations of Sr. There was a significant positive correlation between Sr and calcium (Ca), and between barium (Ba) and Ca. Ca, Sr, and Ba interact with each other and can exert similar chemical and pharmacological effects. Mean ($n \geq 3$) eggshell:egg ratios for Sr varied with species and ranged from 6.1:1 to 40.2:1; ratios for individual eggs reached 92.7:1. Mean Sr/Ca values ranged from 1.3×10^{-3} to 3.0×10^{-3} and mean eggshell thickness ranged from 83 ± 6 to 120 ± 9 µm for all species. Eggshell thickness was not significantly correlated with Sr for any species but tended to increase with Sr concentrations. We postulate that high concentrations of Sr in the shell could affect later-stage embryos by possible

Manuscript received 7 February 2006; accepted 17 August 2006.

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interference with Ca metabolism and bone growth, resulting in reduced hatching success and potential minor beak deformities.

Key words: *Arizona, birds, ecotoxicology, eggshell, metals, strontium.*

Significado Ecotoxicológico Potencial de Concentraciones Elevadas de Estroncio en Cáscaras de Huevo de Aves Paserinas

Resumen. Investigamos la ocurrencia y el significado ecotoxicológico potencial de concentraciones elevadas de estroncio (Sr) en las cáscaras de huevo de nueve aves paserinas de cuatro regiones en Arizona. Las concentraciones de Sr en las cáscaras variaron entre 70 y 1360 $\mu g\ g^{-1}$ de peso seco (promedio global $= 684 \pm 345$ SD $\mu g\ g^{-1}$ peso seco) para las cuatro regiones. El 23% de las cáscaras tuvieron concentraciones mayores a 1000 $\mu g\ g^{-1}$ de peso seco. Según nuestro conocimiento, estos son los niveles más altos de Sr que se han reportado en cáscaras de huevo de aves en Norte América. De las nueve especies, *Molothrus ater* presentó la concentración de Sr más elevada. Existió una correlación positiva y significativa entre el Sr y el calcio (Ca), y entre el bario (Ba) y el Ca. Estos tres elementos interactúan entre sí, y pueden ejercer efectos químicos y farmacológicos similares. Las proporciones promedio de Sr entre cáscara:huevo variaron dentro de un rango de 6.1:1 a 40.2:1 de acuerdo con la especie; las proporciones observadas en huevos individuales alcanzaron un valor de 92.7:1. Las proporciones medias de Sr/Ca variaron de 1.3×10^{-3} a 3.0×10^{-3} y el grosor medio de la cáscara varió de 83 ± 6 a 120 ± 9 µm para todas las especies. El grosor de la cáscara no se correlacionó significativamente con el Sr en ninguna especie, pero existió una tendencia a incrementar con las concentraciones de Sr. Postulamos que las altas concentraciones de Sr en la cáscara podrían afectar a los embriones durante las últimas etapas del desarrollo a través de una posible interferencia con el metabolismo del Ca y del crecimiento de los huesos, dando como resultado una reducción del éxito de eclosión y, potencialmente, deformidades menores del pico.

Strontium (Sr) is an inorganic element that occurs naturally in igneous rocks, primarily as celestite (strontium sulfate) and strontiamite (strontium carbonate; Ober 1989). There have not been any active celestite mines in the United States since 1959; however, mining of celestite occurred in Texas and California during World War II (Ober 1989). In the United States, Arizona is among the few states where significant celestite deposits have been discovered and elevated concentrations of Sr in stream sediments have been documented in several regions (Theobald and Barton 1988). Inputs of Sr in the environment derive primarily from weathering of underlying soil and rock, but also from atmospheric deposition (Vitousek et al. 1999).

Strontium mobilization in organisms is closely associated with mobilization of calcium (Ca); higher Ca requirements of females during egg production lead to an increase in the absorption of Ca in the gut and a corresponding increase in the absorption of Sr (Kottferova et al. 2001). High concentrations of strontium injected into white leghorn chicken eggs resulted in embryo mortality (Ridgway and Karnofsky 1952). Also, white leghorn hens fed Sr in the diet decreased egg production, and those fed 4% Sr laid an increased number of cracked eggs (Mraz et al. 1967). Strontium apparently interferes with normal metabolism of vitamin D (Moon 1994), which facilitates the transport of Ca from the eggshell to the embryo (Elaroussi and Deluca 1994). Thus, high concentrations of Sr in eggshell could result in increased egg breakage, decreased hatching success, and insufficient transport of Ca for bone formation, which could result in rickets (Neufeld and Boskey 1994) or bill deformities.

Strontium has been routinely reported in bird eggs; there are 3523 records of Sr in wild bird eggs in the Environmental Contaminants Data Management System (ECDMS) of the U.S. Fish and Wildlife Service (Nierwienski 2005). Yet, little information is available on concentrations of Sr in bird eggshells. During 1999 and 2000, elevated concentrations of Sr were found in the egg contents of Yellow Warblers (*Dendroica petechia*) and Song Sparrows (*Melospiza melodia*) and in the egg contents and eggshells of Yellow-breasted Chats (*Icteria virens*) and southwestern Willow Flycatchers (*Empidonax traillii extimus*) in Arizona (Mora 2003, Mora et al. 2003). Concentrations of Sr in the eggshells of Yellow-breasted Chats were three times greater than those in Willow Flycatchers. Mora (2003) speculated that the lower hatching success of Willow Flycatchers could have been associated with high concentrations of Sr in their eggshells. Elevated concentrations of Sr in eggshells of passerine birds from Arizona are probably a result of high deposits in the environment. Mean Sr concentrations of 869 $\mu g\ g^{-1}$ dry weight (dw) were reported in eight carcasses of Sonoran mud turtles (*Kinosternon sonoriense*) found dead at Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona (K. King et al., U.S. Fish and Wildlife Service, unpubl. data). Insects and other invertebrates were the main components in the diet of birds collected in Arizona and were likely an important source of Sr. Concentrations of Sr in insects ranged from 8 to 117 $\mu g\ g^{-1}$ dw (Mora et al. 2003), and were 44 to 230 $\mu g\ g^{-1}$ dw in sediments (Mora 2003).

Over a period of several years, a low rate (1.4%) of bill and eye (87% and 13%, respectively) deformities was reported in adult and nestling Willow Flycatchers from the southwestern United States (M. Sogge and E. Paxton, U.S. Geological Survey, unpubl. data). However, deformities in embryos that may have failed to hatch were not investigated. Other species in the region have not been studied as intensively as the Willow Flycatcher; hence, it is not known whether these deformities also occur in other birds. The objective of this study was to determine if the occurrence of Sr in eggshells is widespread

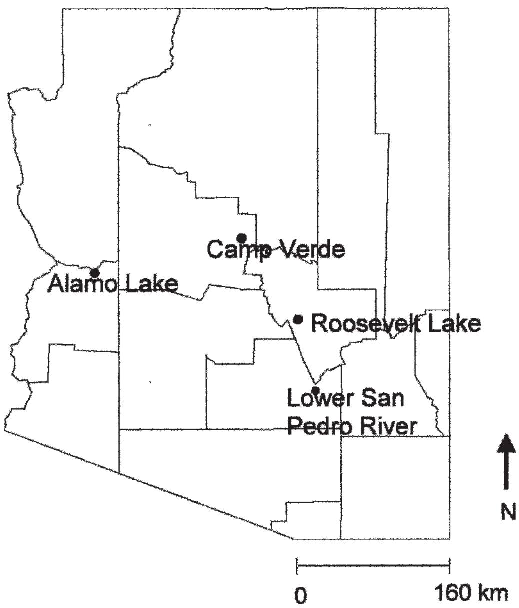

FIGURE 1. Map of Arizona showing sampling locations. The four locations were selected based on a previous study and represent important nesting habitat for the Southwestern Willow Flycatcher.

throughout Arizona and if it is more prevalent in some species than in others. We also evaluated whether Sr affected eggshell thickness and the potential reproductive and fitness consequences of elevated concentrations of Sr in eggshells of passerine birds. Barium (Ba) concentrations in eggshells are also provided because Ba is another element associated with Ca and Sr and can exert similar chemical and pharmacological effects (John et al. 2005).

METHODS

During 2000, we collected eggs of nine bird species nesting at Alamo Lake, Roosevelt Lake, Camp Verde, and the lower San Pedro River in Arizona (Fig. 1). Eggs were collected opportunistically as nests were encountered, or from abandoned nests of known species. Only one egg per nest was collected. Most eggs were from the Vermillion Flycatcher (*Pyrocephalus rubinus*; $n = 5$), Bell's Vireo (*Vireo bellii*; $n = 12$), Yellow Warbler ($n = 6$), and Brown-headed Cowbird (*Molothrus ater*; $n = 7$; Table 1). Eggs were wrapped in aluminum foil and placed in egg cartons or in chemically cleaned glass jars and stored in the refrigerator until chemical analysis.

CHEMICAL ANALYSIS

Egg contents were analyzed separately and the data have been reported elsewhere (Mora 2003). Eggshell samples were washed sparingly with deionized water to remove adhering soil particles and then lyophilized. Dry samples were weighed to the nearest 0.0001 g and transferred to polypropylene digestion vessels, where they were digested with nitric acid, hydrochloric acid, and hydrogen peroxide. Following digestion, samples were diluted to volume using deionized water. Quality control samples processed with the eggshells included reagent blanks, standard reference materials, duplicate samples, and spiked samples. Reagent blanks are laboratory quality control samples that contain all the same acids and water used to process the samples. They are used to estimate contamination from laboratory reagents and glassware. Standard reference materials (SRMs) have known analyte concentrations and are certified by a recognized authority. For this study, we used the SRM "1400 – Bone Ash" produced by the U.S. National Institute of Standards and Technology (NIST), because its composition was similar to that of the samples being analyzed. SRM recovery is used as an indicator of accuracy. Duplicate samples consist of two aliquots of a sample that are prepared and analyzed, and the relative percent difference of their measured values is used to estimate precision (reproducibility). Spiked samples consist of two aliquots of a sample that are prepared and analyzed, one of which is spiked with a known amount of analyte; the recovery of this spike is used to estimate accuracy of the measurement process. Because limited quantities of eggshell samples precluded subsampling for duplicate or spike purposes, laboratory quality control samples obtained from chicken eggshells were prepared and used for these purposes. Digested samples were analyzed by inductively coupled plasma–optical emission spectroscopy. Instruments were calibrated using a blank and three standards and the calibration was verified before sample analysis, after every tenth sample, and at the end of analysis. Emission peaks measured using inductively coupled plasma–optical emission spectroscopy were corrected using off-peak background correction and interelement factors. Concentrations are reported as parts per million ($\mu g\ g^{-1}$) on a dry weight (dw) basis.

EGGSHELL THICKNESS

Eggshells were washed with regular water until the inner shell membrane could be removed from the shell by gently rubbing with one finger. After membrane removal, the eggshells were washed with distilled water, rinsed with acetone, and allowed to dry prior to measuring thickness. We measured eggshell thickness near the equator three times with a micrometer (Starrett, Athol, Massachusetts) and took the average of the three measurements.

STATISTICAL ANALYSES

All statistical analyses were conducted with SAS software (SAS Institute 2003). The data from Bell's Vireos and Yellow Warblers were used to determine differences in concentrations between species within locations, and within species between locations (Roosevelt Lake and the lower San Pedro River). We used *t*-tests to compare concentrations of Ba, Sr, and Ca in the two species and two locations. Differences among three species by location were determined only for Roosevelt Lake. If no differences

TABLE 1. Concentrations (mean ± SD) of barium (Ba; μg g^{-1} dry weight), strontium (Sr; μg g^{-1} dry weight), and calcium (Ca; mg g^{-1}) in eggshells of nine passerine birds from four regions in Arizona. Concentrations of elements in Bell's Vireo and Yellow Warbler eggshells from Roosevelt Lake and San Pedro River were not different; thus, the data were combined by species for statistical analyses. The data were also combined for each location for statistical comparisons. Vermillion Flycatcher eggs were obtained from one location and Brown-headed Cowbird eggs were mostly gathered from Camp Verde. Within columns (among species, and among locations), values not sharing the same letter are significantly different. Eggshell thickness and Sr eggshell/egg relationships were determined by species only. Eggshell thickness was greater in Brown-headed Cowbirds than in most species and Sr concentrations were 6–93 times greater in eggshells than in eggs. Brown-headed Cowbird eggshells had greater concentrations of Ba, Sr, and Ca than eggshells of other species. Eggshells of birds from Camp Verde had greater concentrations of Ba, Sr, and Ca than those of birds from other regions.

	n	Ba	Sr	Ca	Sr/Ca	Eggshell thickness (μm)	Sr (eggshell/egg)[a]
Species							
Vermillion Flycatcher (*Pyrocephalus rubinus*)	5	50 ± 24 AB	368 ± 160 B	269 ± 33 B	0.0013	93 ± 31 B	11.9
Bell's Vireo (*Vireo bellii*)	12	40 ± 27 B	643 ± 384 AB	297 ± 29 AB	0.0021	93 ± 11 B	40.2
Black-throated Gray Warbler (*Dendroica nigrescens*)	1	40	418	285	0.0015	90	83.6
Yellow Warbler (*Dendroica petechia*)	6	48 ± 25 AB	814 ± 297 AB	291 ± 55 AB	0.0027	83 ± 6 B	6.1
Common Yellowthroat (*Geothlypis trichas*)	1	32	472	272	0.0017	ND[b]	67.4
Summer Tanager (*Piranga rubra*)	1	40	834	319	0.0026	120	92.7
Song Sparrow (*Melospiza melodia*)	3	43 ± 27 AB	634 ± 128 AB	326 ± 21 AB	0.0019	107 ± 6 AB	10.1
Brown-headed Cowbird (*Molothrus ater*)	7	88 ± 42 A	1002 ± 242 A	335 ± 19 A	0.0030	120 ± 9 A	29.5
Lesser Goldfinch (*Carduelis psaltria*)	1	26	1080	350	0.0031	110	72.0
Location							
Alamo Lake	2	45 ± 15 AB	919 ± 242 AB	326 ± 27 AB	0.0028		
Camp Verde	7	88 ± 40 A	959 ± 229 A	343 ± 16 A	0.0028		
Roosevelt Lake	17	39 ± 20 B	498 ± 197 B	276 ± 36 B	0.0018		
San Pedro River	11	48 ± 29 B	832 ± 414 A	314 ± 17 B	0.0026		

[a] Mean Sr values for egg contents were obtained from Mora (2003).
[b] Not determined.

in concentrations of Ba, Sr, and Ca were observed among the three species at Roosevelt Lake or between Bell's Vireos and Yellow Warblers at the lower San Pedro River, the data from all species were combined (by element) for each location for further comparisons. If no significant differences were observed by species (Bell's Vireos and Yellow Warblers) between locations (Roosevelt Lake and the lower San Pedro River), data for each species from the four locations were combined for species comparisons. Differences among species within locations, among species from all locations, and among locations for all species combined were determined by GLM ANOVA. GLM ANCOVA was used to determine linear relationships between Ba and Sr with Ca, and between eggshell thickness and Sr, considering the simultaneous effects of species and location. If the interactions were not significant, we used a standard ANCOVA. Type III sums of squares and *F*-statistics were used to determine the significance of each variable. Simple linear regression was used to determine relationships between eggshell thickness and Sr concentrations by species and between Sr/Ca and Sr. Values are reported as means ± SD. The level of significance was set at $P < 0.05$.

RESULTS

Concentrations of Ba, Ca, and Sr were not significantly different among eggshells of Bell's Vireos, Yellow Warblers, and Vermillion Flycatchers at Roosevelt Lake, or between eggshells of Bell's Vireos and Yellow Warblers at the lower San Pedro River. Mean concentrations of Ba, Ca, and Sr were significantly greater in eggshells of birds from Camp Verde than in those from Roosevelt Lake (ANOVA, $F_{3,33} = 5.4$, 5.9, and 11.3, all $P < 0.01$; Table 1). Ba and Ca concentrations were also greater in eggshells from Camp Verde than in those from the lower San Pedro River (ANOVA, $F_{3,33} = 5.4$ and 11.3, respectively, both $P < 0.01$), but Sr concentrations were similar between these two locations. Concentrations of Ba, Sr, and Ca in eggshells of birds from Alamo Lake were not different from those in any of the other three locations; however, only two samples from Alamo Lake were analyzed.

Brown-headed Cowbird eggshells had significantly higher concentrations of Ba than Bell's Vireo eggshells (ANOVA, $F_{4,28} = 3.0$, $P = 0.03$), and significantly higher concentrations of Sr ($F_{4,28} = 3.6$, $P = 0.02$), and Ca ($F_{4,28} = 3.4$, $P = 0.02$) than Vermillion Flycatchers, but concentrations of all elements were similar to those in Yellow Warbler and Song Sparrow eggshells (Table 1). Eggshell thickness was significantly greater in Brown-headed Cowbirds than in Bell's Vireos, Vermillion Flycatchers, and Yellow Warblers (ANOVA, $F_{4,28} = 5.9$, $P < 0.01$).

Neither species nor location had a significant effect on the relationship between Ba and Sr with Ca. Therefore, there was a significant positive relationship between Sr and Ca ($R^2 = 0.46$, $P = 0.009$; Fig. 2) and between Ba and Ca ($R^2 = 0.26$, $P = 0.003$). Eggshell thickness was not significantly correlated with Ca or Sr in the Vermillion Flycatcher ($R^2 = 0.09$ and 0.07, $P = 0.91$ and 0.93), Bell's Vireo ($R^2 = 0.13$ and 0.14, $P = 0.53$ and 0.50), Yellow Warbler ($R^2 = 0.42$ and 0.66, $P = 0.44$ and 0.19), or Brown-headed Cowbird ($R^2 = 0.07$ and 0.17, $P = 0.86$ and 0.68). Variation in eggshell thickness was explained more by species than by Sr concentrations (ANCOVA, $F_{5,27} = 5.3$, $P < 0.001$). There was a highly significant relationship between Sr/Ca and Sr ($R^2 = 0.95$, $P < 0.001$; Fig. 3). We estimated the eggshell/egg values for Sr by using mean egg content concentrations from a previous study (Mora 2003). The eggshell/egg values varied from 6.1 in Yellow Warblers to 92.7 in one Summer Tanager egg (Table 1).

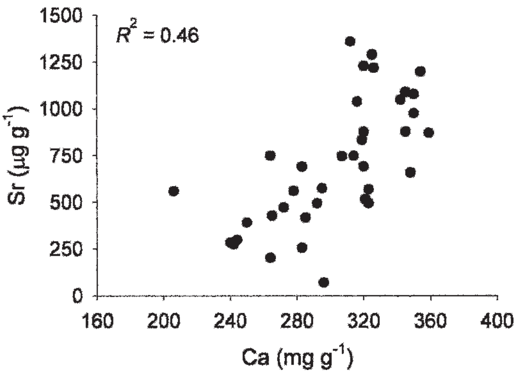

FIGURE 2. Relationship between Ca and Sr in eggshells of nine passerines from four locations in Arizona. Each circle represents an individual value. Accumulation of Sr is strongly correlated with Ca levels in the eggshell; birds with the greatest Ca levels also had the greatest concentrations of Sr.

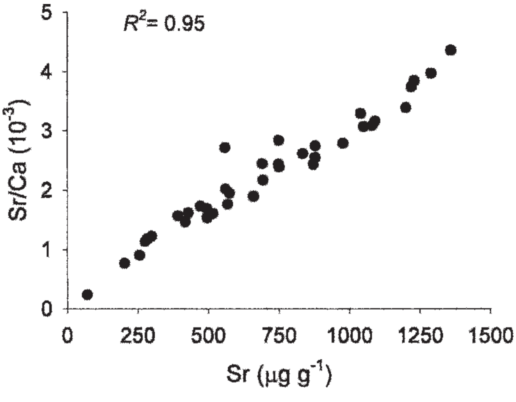

FIGURE 3. Relationship between Sr and Sr/Ca values in eggshells of nine passerines from four locations in Arizona. Each circle represents an individual value. Sr concentrations were highly correlated with Sr/Ca values, suggesting that Sr varies with Ca concentrations in the eggshell.

DISCUSSION

Our study provides data in support of the eggshell as an important excretion reservoir of nonessential inorganic elements (particularly Sr) for female birds. To date, not many studies have reported concentrations of inorganic elements in eggshells and very few have recognized that most female birds sequester various heavy metals in eggshells (Mraz et al. 1967, Burger 1994, Morera et al. 1997, Dauwe et al. 1999, Mora 2003, Lam et al. 2004, Agusa et al. 2005). Our results strongly suggest that Sr is highly likely to accumulate in eggshell, particularly in regions with unusually high concentrations of Sr in the environment, such as Arizona (Theobald and Barton 1988).

The biological and toxicological significance of elevated concentrations of Sr in eggshells of wild birds is not well known. Mobilization of Sr in the eggshell during the latter half of incubation could affect embryo development (Elaroussi and Deluca 1994), reduce eggshell strength (Mraz et al. 1967), and inhibit the active transport of eggshell Ca to the chorioallantoic membrane (Moon 1994). Embryos of Japanese Quail (*Coturnix japonica*) deficient in Vitamin D did not hatch because they could not obtain Ca from the shell (Elaroussi and Deluca 1994). Thus, one potential consequence of elevated concentrations of Sr in the eggshell is interference with Ca transfer from the eggshell to the embryo, leading to insufficient Ca for bone formation, and resulting in rickets (Neufeld and Boskey 1994) or other deformities not yet studied in the laboratory. $Strontium^{2+}$ administered to rats on low Ca^{2+} diets decreased bone growth and inhibited the bone calcification process (Matsumoto 1976). Low rates of bill and eye deformities were observed in adult and nestling Willow Flycatchers in Arizona during 1996–2000 (M. Sogge and E. Paxton, U.S. Geological Survey, unpubl. data). Concentrations of Sr in eggshells were elevated (Mora 2003, Mora et al. 2003); however, concentrations of the most persistent contaminants that are known to be associated with deformities were low in Willow Flycatcher eggs.

A few studies have reported elevated concentrations of Sr in wildlife (Hothem and Powell 2000, Hui et al. 2002; C. Day et al., U.S. Fish and Wildlife Service, unpubl. data; C. Thomas, U.S. Fish and Wildlife Service, unpubl. data). However, to our knowledge, there are no other studies in the literature that suggest that Sr in eggs or eggshells of wild birds could have adverse effects during embryo development. Schwarzbach et al. (2006) reported Sr concentrations as high as 176 $\mu g\ g^{-1}$ dw in unhatched eggs of California Clapper Rails (*Rallus longirostris obsoletus*) from San Francisco Bay. Clapper Rails had low hatchability and there was a positive association between high concentrations of Sr and embryo deformities. Additionally, there was an increase in Sr concentration in eggs throughout development. Three deformed embryos had mean values of Sr of 121.4 $\mu g\ g^{-1}$ dw, whereas the mean for normal embryos was 66.1 $\mu g\ g^{-1}$ dw (Schwarzbach et al. 2006). However, despite these associations with Sr, the reduced hatchability was attributed to elevated concentrations of mercury (Hg), whereas chromium (Cr) and Ba were linked to the observed deformities (shortened feet, polydactyly, and abnormal wings).

Using eggshell/egg values for Sr (range = 6–40) generated in our study, the amount of Sr in the eggshells of the Clapper Rail eggs with deformed embryos would have been between 726 and 4840 $\mu g\ g^{-1}$ dw, at least as high as or greater than concentrations reported in our study. It appears that the proportion of Sr in eggs could be influenced by the stage of embryo development (Schwarzbach et al. 2006) and that as more Ca is needed for bone formation, more Sr is also transferred to the egg contents from the eggshell, thus influencing the eggshell:egg ratios. More data are needed to better document the proportion of Sr that accumulates in the eggshell and the potential negative implications of such accumulation.

We appreciate the field assistance given by biologists of the Arizona Game and Fish Department and Texas A&M University. H. Yard provided the eggs from Camp Verde and B. Nierwienski provided the data for Sr from the Environmental Contaminants Data Management System (ECDMS). This manuscript was greatly improved by comments from D. Anderson, D. Hoffman, S. Schwarzbach, *The Condor*'s editorial staff, and an anonymous reviewer.

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