Ecotoxicology (2011) 20:1467–1475
DOI 10.1007/s10646-011-0703-4

Regional and interspecific variation in Sr, Ca, and Sr/Ca ratios in avian eggshells from the USA

Miguel A. Mora · Bryan Brattin · Catherine Baxter · James W. Rivers

Accepted: 9 May 2011 / Published online: 10 June 2011

Abstract To examine regional variation in strontium (Sr), which at high concentrations may reduce eggshell quality, increase egg breakage and reproductive failure, we analyzed Sr, and calcium (Ca) concentrations and Sr/Ca ratios in eggshells from 20 avian species from California, Texas, Idaho, Kansas, and Michigan. In addition, we included data previously reported from Arizona to expand the regional comparisons and to better establish patterns of Sr, and Sr/Ca ratios in bird species across the United States. We found Sr concentrations varied significantly among regions, among species, and among foraging guilds; this variability is strongly influenced by the Sr/Ca ratios in surface water from locations close to the region where the eggshells were collected. Sr concentrations and Sr/Ca ratios were significantly higher in bird eggshells from the Volta wildlife region in the San Joaquin Valley, California and in various locales from Arizona. Sr concentrations and Sr/Ca ratios in bird eggshells from other locations in the USA were lower than those detected in these two regions. Among foraging guilds, invertivores had the highest Sr concentrations and Sr/Ca ratios and carnivores had the lowest. In general, the Sr/Ca ratio increased strongly with increasing Sr concentrations ($R^2 = 0.99$, $P < 0.0001$). There was a significant correlation ($R^2 = 0.58$, $P < 0.0001$) between Sr/Ca ratios in water and the average Sr/Ca ratios in eggshells suggesting that these values could be determined from Sr/Ca ratios in water. Eggshell thickness was poorly correlated with Sr ($R^2 = 0.03$) but had a significant and positive correlation with Ca and was more properly correlated by a quadratic equation ($R^2 = 0.50$, Thickness $= 2.13 - 0.02\text{Ca} - 3.07 * 10^{-5}\text{Ca}^2$). Our study provides further evidence that Sr accumulates significantly in the avian eggshell, in some regions at concentrations which could be of concern for potential negative effects on reproduction. We suggest that when assessing the effects of metals on avian reproduction in regions with high Sr deposits in rock and soil, Sr concentrations in the eggshell also should be measured to evaluate additional effects on thickness and reproduction.

Keywords Avian eggshell · Calcium (Ca) · Strontium (Sr) · Sr/Ca ratios

Introduction

Strontium (Sr) is an inorganic element that occurs naturally in igneous rocks (Ober 1989) and is often associated with calcium (Ca) deposits. Sr is chemically similar to Ca and is often incorporated by organisms into bone and eggshells. Sr mobilization in organisms is associated with mobilization of Ca; thus, higher Ca requirements of females during egg production result in an increased uptake of Ca and Sr (Kottferová et al. 2001), particularly in areas with elevated

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M. A. Mora (✉) · C. Baxter
Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258, USA
e-mail: mmora@tamu.edu

B. Brattin
Trace Element Research Laboratory, Texas A&M University, College Station, TX 77843, USA

J. W. Rivers
Department of Forest Ecosystems & Society, Oregon State University, Corvallis, OR 97331-5752, USA

Present Address:
B. Brattin
Department of Environmental and Radiological Health Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523-9001, USA

concentrations of Sr. Typically, Ca and Sr incorporated into the egg originates from the bones and blood of the female (Quintana et al. 1980). Shell formation takes place largely in the uterus (Burley and Vadehra 1989), where minerals are extracted from the blood. Experiments with Sr and Ca mixtures in laying hens have shown that the barrier between the blood and the forming shell has a Sr/Ca discrimination ratio of 92% (Simkiss 1967); proportionally, more Ca than Sr is incorporated into the shell.

Understanding Sr accumulation in the egg and eggshell is important for several reasons. First, Sr interferes with vitamin D metabolism (Moon 1994) and with Ca transport to the embryo (Elaroussi and Deluca 1994). The inclusion of large amounts of Sr in the diet (20 g/kg $SrCl_2$ per day for 6 days) results in the cessation of egg laying and significant structural changes in the eggshell (Quintana et al. 1980). Among these changes are the alteration of the crystal form of the calcite columns, thinning of the shell along with uneven thickness, and general homogenization (disappearance) of distinct shell layers (Quintana et al. 1980). Sr is incorporated into the inorganic (e.g. calcite columns, or palisade layer) and organic (e.g. mammillary cores) layers of the shell (Quintana et al. 1980).

Strontium has been routinely reported in wild bird eggs, yet little information is available on concentrations of Sr in bird eggshells. Previously, we estimated that concentrations of various inorganic elements (As, Ba, Cu, Ni, Pb, Sr, and V) were 2–35 times greater in eggshells than in eggs of yellow-breasted chats (*Icteria virens*) from Arizona (Mora 2003). Subsequently, the eggshell/egg ratio for Sr was further estimated for other avian species and ranged from 6 to 93 (Mora et al. 2007). Schwarzbach et al. (2006) observed a significant correlation between relatively high concentrations of Sr in egg contents and embryo deformities of clapper rails (*Rallus longirostris obsoletus*) from San Francisco Bay, California. Schwarzbach et al. (2006) also reported an increased concentration of Sr in eggs throughout development, suggesting transfer of Sr from the eggshell to the egg. Between 1996 and 2000, bill malformations were reported in southwestern willow flycatchers (*Empidonax traillii extimus*, Sogge and Paxton, U.S. Geological Survey, unpubl. data) from various regions in Arizona. Contaminant studies undertaken to determine the cause of such bill malformations reported high concentrations of Sr in the avian eggshell, which could have been associated with reduced hatching success in some regions, particularly in Roosevelt Lake (Mora 2003; Mora et al. 2007). High concentrations of Sr in the bird eggshell could result in increased egg breakage (Mraz et al. 1967) and in competition with Ca transport to the embryo, leading potentially to rickets (Neufeld and Boskey 1994) and possibly bone deformities.

In the United States, Arizona is one of the few states where significant celestite deposits rich in Sr have been discovered and elevated concentrations of Sr in stream sediments have been documented in several locations (Theobald and Barton 1988). Accordingly, the highest concentrations of Sr have been reported in the southwest, including Arizona, New Mexico, western Oklahoma, and northern Texas and the lowest concentrations in the Pacific northwest, southeastern United States, the upper Great Lakes, and most of the east coast (Sillen and Kavanagh 1982). Inputs of Sr in the environment derive primarily from weathering of underlying soil and rock, but also from atmospheric deposition (Vitousek et al. 1999).

Most of the Sr studies in birds have been with poultry. Chickens are very sensitive to some chemicals (i.e. dioxins and dioxin-like compounds; Rice et al. 2003), but rather insensitive to others (i.e., DDT). In the case of Sr, chickens can tolerate and incorporate high concentrations in the eggshell without any negative effects (Doberenz et al. 1969; Quintana et al. 1980). However, very few Sr studies have been replicated with wild birds. Because of the discrimination against Sr in favor of Ca, Sr/Ca ratios also could be used to predict dietary sources and position in terrestrial food chains (Elias et al. 1982; Sillen and Kavanagh 1982). This effect is better known as “biopurification” or the preferential assimilation of Ca relative to Sr, which suggests that Sr/Ca ratios decrease with ascending trophic position in the food web (Burton et al. 1999). A biopurification factor is determined as the ratio in Sr/Ca between the nutrient and the consumer (Elias et al. 1982). Sr/Ca ratios could also be used to determine potential toxicity or negative effects of Sr on embryo development and hatchability in wild birds. Our study had the following objectives; (1) To determine if concentrations of Sr, Ca, and Sr/Ca ratios in the eggshells of various species of passerine and non-passerine birds from several regions in the United States were species specific, varied with region, or by foraging guild; (2) to identify regions and species with elevated Sr concentrations in eggshells; and (3) to determine if Sr/Ca ratios in surface water from nearby regions could be used to predict Sr/Ca ratios in avian eggshells.

Materials and methods

Sample collection

Eggshells ($n = 110$) were obtained from 20 avian species (six foraging guilds) from various locations in California, Texas, Idaho, Kansas, and Michigan between 1985 and 2007 (see Table 1). We also included data previously reported from Arizona (27 eggshells from four species; Mora 2003; Mora et al. 2007), to expand the regional comparisons and to better establish patterns of Sr and Ca

Table 1 Mean ± SD concentrations of Sr and Ca, and Sr/Ca ratios in avian eggshells from selected regions in the USA[a,b]

State	Location	Common name	Family	*n*	Thickness (μm)	Sr (μg/g)	Ca (mg/g)	Sr/Ca (10^{-3})
CA	Edwards AFB	Black-crowned night-heron (*Nycticorax nycticorax*)	Ardeidae	5	276 ± 24	356 ± 151^{D}	339 ± 7^{AB}	1.05 ± 0.43^{CD}
CA	Volta Wildlife Area	Black-necked stilt (*Himantopus mexicanus*)	Recurvirostridae	5	236 ± 15	2666 ± 372^{A}	338 ± 6^{AB}	7.89 ± 1.07^{A}
AZ	Roosevelt Lake	Vermilion Flycatcher (*Pyrocephalus rubinus*)	Tyrannidae	5	88 ± 23	368 ± 160^{D}	269 ± 33^{E}	1.34 ± 0.48^{CD}
AZ	Roosevelt Lake	Bell's vireo	Vireonidae	6	92 ± 10	464 ± 174^{D}	280 ± 29^{CDE}	1.64 ± 0.54^{B}
AZ	Roosevelt Lake	Yellow warbler	Parulidae	4	83 ± 5	654 ± 199^{C}	270 ± 57DE	2.43 ± 0.55^{B}
AZ	Lower San Pedro River	Bell's vireo (*Vireo bellii*)	Vireonidae	5	90 ± 14	768 ± 500^{B}	307 ± 12^{CDE}	2.46 ± 1.57^{B}
AZ	Lower San Pedro River	Yellow warbler (*Dendroica petechia*)	Parulidae	2	80 ± 0	1135 ± 120^{B}	334 ± 11DE	3.41 ± 0.48^{B}
AZ	Camp Verde	Brown-headed cowbird (*Molothrus ater*)	Icteridae	5	112 ± 19	994 ± 226^{BC}	341 ± 19^{AB}	2.92 ± 0.69^{B}
TX	Bryan	Cattle egret (*Bubulcus ibis*)	Ardeidae	5	238 ± 16	345 ± 118^{D}	340 ± 2^{AB}	1.02 ± 0.35^{D}
TX	Lower Rio Grande Valley	Aplomado falcon (*Falco femoralis septentrionalis*)	Falconidae	5	282 ± 27	98 ± 50^{D}	333 ± 10^{ABC}	0.29 ± 0.15^{D}
ID	Deer Flat NWR	Western grebe (*Aechmophorus occidentalis*)	Podicipedidae	5	406 ± 35	252 ± 24^{D}	345 ± 6^{AB}	0.73 ± 0.07^{D}
ID	Bear Lake NWR	Great blue heron (*Ardea Herodias*)	Ardeidae	5	388 ± 43	206 ± 43^{D}	350 ± 4^{A}	0.59 ± 0.12^{D}
ID	Bear Lake NWR	White-faced ibis (*Plegadis chihi*)	Threskiornithidae	5	324 ± 23	558 ± 394^{B}	341 ± 8^{AB}	1.63 ± 1.13^{B}
ID	Bear Lake NWR	Franklin's gull (*Larus pipixcan*)	Laridae	5	270 ± 14	409 ± 59^{D}	330 ± 14^{ABCD}	1.24 ± 0.18^{CD}
KS	Konza Prairie BS	Eastern phoebe (*Sayornis phoebe*)	Tyrannidae	6	100 ± 5	427 ± 107^{D}	313 ± 12^{ABCD}	1.37 ± 0.35^{CD}
KS	Konza Prairie BS	Bell's vireo	Vireonidae	6	87 ± 12	197 ± 97^{D}	317 ± 12^{CDE}	0.62 ± 0.28^{D}
KS	Konza Prairie BS	Barn swallow (*Hirundo rustica*)	Hirundinidade	6	98 ± 7	342 ± 199^{D}	305 ± 10^{BCDE}	1.11 ± 0.63^{CD}
KS	Konza Prairie BS	Gray catbird (*Dumetella carolinensis*)	Mimidae	6	142 ± 9	258 ± 101^{D}	318 ± 8^{ABCD}	0.81 ± 0.32^{D}
KS	Konza Prairie BS	Brown thrasher (*Toxostoma rufum*)	Mimidae	6	154 ± 6	362 ± 176^{D}	321 ± 3^{ABCD}	1.13 ± 0.54^{CD}
KS	Konza Prairie BS	Dickcissel (*Spiza Americana*)	Cardinalidae	6	122 ± 8	381 ± 136^{D}	313 ± 4^{ABCD}	1.21 ± 0.42^{CD}
KS	Konza Prairie BS	Red-winged blackbird (*Agelaius phoeniceus*)	Icteridae	6	119 ± 9	471 ± 163^{D}	321 ± 8^{ABCD}	1.47 ± 0.49^{CD}
KS	Konza Prairie BS	Eastern meadowlark (*Sturnella magna*)	Icteridae	6	146 ± 12	432 ± 288^{D}	313 ± 3^{ABCD}	1.38 ± 0.93^{CD}
KS	Konza Prairie BS	Common grackle (*Quiscalus quiscula*)	Icteridae	6	166 ± 10	491 ± 209^{D}	323 ± 4^{ABCD}	1.51 ± 0.62^{D}
KS	Konza Prairie BS	Brown-headed cowbird	Icteridae	6	146 ± 10	474 ± 138^{D}	328 ± 4^{AB}	1.45 ± 0.43^{CD}
MI	Detroit	Peregrine falcon (*Falco peregrinus anatum*)	Falconidae	5	362 ± 47	39 ± 10^{D}	347 ± 19^{A}	0.11 ± 0.02^{D}
MI	Kalamazoo River	Great-horned owl (*Bubo virginianus*)	Strigidae	5	380 ± 23	45 ± 15^{D}	344 ± 11^{AB}	0.13 ± 0.04^{D}

[a] Within columns (Sr, Ca, and Sr/Ca), values not sharing the same letter are significantly different

[b] Data from Arizona were published previously (Mora 2003; Mora et al. 2007)

concentrations and Sr/Ca ratios in birds across the United States. Of the 22 species examined, eggshells of the Bell's vireo (*Vireo bellii*) and brown-headed cowbird (*Molothrus ater*) were collected from both Arizona and Kansas, and eggshells of the Bell's vireo and yellow warbler (*Dendroica petechia*) were collected from more than one location in

Arizona. The remaining eggshells were exclusive to one species at one location (7 of 12 locations).

After collection, eggshells were washed with tap water removing the outer shell membrane, then 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. Thickness was measured in the presence of the inner shell membrane.

Chemical analysis

Eggshells were analyzed for inorganic elements including Ca and Sr by inductively coupled plasma-optical emission spectroscopy (ICP-OES) as described in Mora et al. (2007). Briefly, eggshells were first rinsed with double deionized water, followed by acetone, to remove dirt and any remnants of egg contents. After air drying, approximately 0.1 g of each eggshell was digested by adding nitric acid, hydrogen peroxide, and hydrochloric acid, heating up to 95°C after each addition. After cooling, samples were diluted with deionized water to 10 ml. Samples were analyzed in sets of 20, and each set included quality control samples (reagent blanks, standard reference material, duplicate samples, and spiked samples). Material for the duplicate and spiked samples (three sets of each type) was domestic chicken eggs. The reagent blanks consisted solely of the same chemicals used to digest the samples and water used for dilution. “SRM 1400 Bone Ash” obtained from National Institute of Standards and Technology (NIST) was used as the standard reference material due to its compositional similarity to the eggshells. One aliquot per set of the chicken eggshell and reagent blank was spiked with 0.1 ml each of three spike solutions of known composition. Samples were analyzed by ICP-OES on a Spectro CirOS instrument. The instrument was calibrated using blanks and external standards, and a rare earth element, ytterbium was used as an internal standard to compensate for physical matrix effects. Calibration curves were checked after every ten samples, and at the end of the analytical sequence. Off-peak background correction and inter-element correction factors were used to compensate for baseline changes and spectral overlap, and cesium chloride was used as an ionization buffer to compensate for “easily ionizable element” effects. Concentrations are reported as ppm (μg/g) on a dry weight basis.

Statistical analyses

All statistical analyses were conducted with the use of SAS software (SAS Institute 2003). We examined differences in Sr and Ca concentrations among species within three locations (Roosevelt Lake, AZ, Bear Lake NWR, ID, and Konza Prairie Nature Preserve, KS) from which there were 15 or more samples from at least three species, by analysis of variance (PROC GLM). For all the samples, there were no significant differences in Sr, Ca, or Sr/Ca ratios among years; thus, we also used ANOVA to determine differences among species and locations with years combined. However, because location and species position in the food chain are known to influence Sr accumulation in birds; we grouped species by foraging guilds (carnivores, herbivores, insectivores, invertivores, omnivores, and piscivores) and conducted an analysis of covariance, with foraging guild as the categorical variable and location, represented by Sr/Ca ratios in surface water taken from a previous study, as the continuous covariate. Invertivores were classified as species feeding primarily on aquatic invertebrates to separate them from the more strictly terrestrial insectivore species. Type III sum of squares and F statistics were used to determine significance. The LSMEANS comparison procedure with the Tukey adjustment was used to determine which means were significantly different. Simple linear regression was used to determine relationships between eggshell thickness and Sr and Ca concentrations by species, between Sr/Ca and Sr, and between Sr/Ca ratios in avian eggshells and published Sr/Ca ratios in water (Sillen and Kavanagh 1982) from the closest locations to our sampling sites. The relationship between eggshell thickness and Ca was more properly assessed by a polynomial quadratic equation. Based on the assumption that the Sr/Ca ratios in watercourses tend to remain constant within a major river or lake drainage system (Sillen and Kavanagh 1982), we used average Sr/Ca ratios of water samples collected in the 1960s in regions close to the areas where the bird eggshells were collected to determine if Sr/Ca ratios in water could be used to predict Sr/Ca ratios in avian eggshells. Values are reported as means ± SD. The level of significance was set at $P < 0.05$.

Results

There were no significant differences in Sr concentrations or Sr/Ca ratios among species at each of three locations (Roosevelt Lake, AZ, Bear Lake NWR, ID, and Konza Prairie Nature Preserve, KS) from which there were 15 or more samples from at least three species. However, when all the species from all locations were compared, mean Sr and Ca concentrations and Sr/Ca ratios in avian eggshells varied by species and ranged between 39 and 2,666 μg/g dw for Sr; 269 and 350 mg/g for Ca; and between 0.11 and 7.89 for Sr/Ca (Table 1). There were significant interspecific differences in Sr ($F_{21,115} = 25.30$, $P < 0.0001$) and Ca concentrations ($F_{21,115} = 7.41$, $P < 0.001$), and Sr/Ca ratios ($F_{21,115} = 24.66$, $P < 0.0001$, Table 1). Also, both

Sr ($F_{11,125} = 60.96$, $P < 0.0001$) and Ca ($F_{11,125} = 20.21$, $P < 0.0001$) concentrations and Sr/Ca ratios ($F_{11,125} = 57.26$, $P < 0.0001$) were significantly different among locations (Table 2).

Both Sr and Sr/Ca ratios were significantly higher in black-necked stilts (*Himantopus mexicanus*) from the Volta region in California than in the rest of the species from other locations (Fig. 1; Table 1). Yellow warblers from the Lower San Pedro River and brown-headed cowbirds from Camp Verde in Arizona had the second and third highest concentrations of Sr and Sr/Ca, respectively, and also were significantly different from most of the other species. Generally, concentrations of Sr and Sr/Ca ratios were not different among the rest of the species (Table 1). Among locations, concentrations of Sr and Sr/Ca ratios were significantly greater in birds from the Volta Wildlife Area in California than in the rest of the locations (Table 2). Birds from the Lower San Pedro River and Camp Verde, Arizona had the second highest Sr concentration and Sr/Ca ratios, whereas birds from Michigan had the lowest. Ca concentrations did not follow the same pattern as Sr concentrations. The lowest Ca values were observed in vermilion flycatchers (*Pyrocephalus rubinus*), yellow warblers, and Bell's vireos from Roosevelt Lake in Arizona, whereas most other species from other regions had higher values (Tables 1, 2).

Comparisons among species grouped by foraging guilds showed significant differences in concentrations of Sr ($F_6 = 5.4$, $P < 0.0001$); Ca ($F_6 = 571.4$, $P < 0.0001$), and Sr/Ca ratios ($F_6 = 5.1$, $P < 0.0001$). The interaction between Sr/Ca ratios in water and foraging guild was significant for Ca ($F_6 = 11.8$, $P < 0.0001$), Sr ($F_6 = 39.3$, $P < 0.0001$), and Sr/Ca ($F_6 = 38.7$, $P < 0.0001$), indicating that the significant differences in Ca, Sr, and Sr/Ca concentrations among foraging guilds were affected by the variation in Sr/Ca ratios in surface water from each region (Table 3). However, except for the insectivore group, Ca concentrations did not vary among other foraging guilds nor were they influenced by regional Sr/Ca ratios in water. The negative slope (−16.61) for the interaction Sr/Ca water * foraging guild (insectivores) was significant ($P < 0.001$) suggesting that Ca values in the insectivore group tended to decrease as the Sr/Ca ratios in surface water increased. For Sr, the *P*-values for the slopes of the regression lines representing the interaction Sr/Ca in water and herbivore, insectivore, and invertivore guilds were significant ($P = 0.0003$, 0.0109, and <0.0001, respectively) indicating that the Sr/Ca ratios in water had a significant effect on Sr concentrations in these guilds. Accordingly, Sr concentrations tended to increase as Sr/Ca ratios in water increased, particularly in invertivores. There were no effects of Sr/Ca ratios in water on Sr values in omnivores, piscivores, and carnivores. Sr/Ca ratios in eggshells provided the same results as Sr. Concentrations of Sr and Sr/Ca ratios were greater in eggshells of invertivores (feeding primarily on aquatic invertebrates) than in the rest of the other groups (Table 4). Herbivores had the second highest concentrations followed by insectivores and omnivores. Carnivores, comprised by the great horned owl (*Bubo virginianus*) and peregrine falcon (*Falco peregrinus anatum*) from Michigan, and aplomado falcon (*Falco femoralis septentrionalis*) from Texas, had the lowest concentrations of Sr and Sr/Ca ratios. Mean Sr concentrations in birds from the Volta region (black-necked stilts) in California were over 65 times greater than those in birds from Michigan. In general, the analysis of covariance suggests that the type of species, foraging guild, and location (reflected by Sr/Ca in water), all have an effect on Sr accumulation in the eggshell.

Table 2 Mean ± SD concentrations of Sr and Ca, and Sr/Ca ratios in avian eggshells grouped by location[a]

State	Location	*n*	Sr (μg/g dw)	Ca (mg/g dw)	Sr/Ca (10^{-3})
Arizona	Lower San Pedro River	7	873 ± 448^{B}	315 ± 17^{B}	2.73 ± 1.4^{B}
	Camp Verde	5	994 ± 226^{B}	341 ± 19^{ABC}	2.92 ± 0.7^{B}
	Roosevelt Lake	15	482 ± 199^{C}	273 ± 37^{D}	1.75 ± 0.7^{C}
California	Edwards Air Force Base	5	356 ± 151^{CDE}	339 ± 7^{ABC}	1.05 ± 0.4^{CDE}
	Volta Wildlife Area	5	2666 ± 372^{A}	338 ± 6^{ABC}	7.89 ± 1.1^{A}
Idaho	Deer Flat NWR	5	252 ± 24^{CDE}	345 ± 6^{A}	0.73 ± 0.1^{CDE}
	Bear Lake NWR	15	391 ± 261^{CD}	340 ± 12^{A}	1.15 ± 0.8^{CDE}
Kansas	Konza Prairie Nature Preserve	60	383 ± 183^{CD}	317 ± 9^{C}	1.21 ± 0.6^{CD}
Michigan	Detroit	5	39 ± 10^{E}	347 ± 19^{A}	0.11 ± 0.02^{E}
	Kalamazoo River	5	45 ± 15^{E}	344 ± 10^{A}	0.13 ± 0.04^{E}
Texas	Bryan	5	345 ± 118^{CDE}	340 ± 2^{ABC}	1.02 ± 0.3^{CDE}
	Lower Rio Grande Valley	5	98 ± 50^{DE}	333 ± 10^{ABC}	0.29 ± 0.1^{DE}

[a] Within columns, values not sharing the same letter are significantly different

Fig. 1 Map of the United States showing the locations from where the eggshells were collected and the locations from where the surface water samples were taken. Values and locations for water were taken from Sillen and Kavanagh (1982) and Skougstad and Horr (1963)

Table 3 ANCOVA results (type III sum of squares) of the analysis of Sr, Ca, and Sr/Ca ratios in avian eggshells from selected regions in the USA, grouped by foraging guilds

Source	DF	Type III SS	Mean square	F value	Pr > F
Model 1: Ca = forguild + SrCaW * forguild					
Forguild	6	933139.11	155523.19	571.38	<.0001
SrCaW * forguild	6	19321.97	3220.33	11.83	<.0001
Model 2: Sr = forguild + SrCaW * forguild					
Forguild	6	1680908.65	280151.44	5.39	<.0001
SrCaW * forguild	6	12261019.90	2043503.32	39.33	<.0001
Model 3: SrCa = forguild + SrCaW * forguild					
Forguild	6	14.59	2.43	5.08	0.0001
SrCaW * forguild	6	110.96	18.49	38.66	<.0001

Table 4 Mean ± SD concentrations of Sr and Ca, and Sr/Ca ratios in avian eggshells from selected regions in the USA, grouped by foraging guilds[a]

Foraging guilds	*n*	Sr (μg/g dw)	Ca (mg/g dw)	Sr/Ca (10^{-3})
Herbivores	11	711 ± 322^{B}	333 ± 14^{B}	2.12 ± 0.93^{B}
Invertivores	10	1612 ± 1168^{A}	339 ± 6.8^{B}	4.7 ± 3.45^{A}
Insectivores	64	442 ± 276^{B}	305 ± 26.5^{A}	1.4 ± 0.90^{BC}
Omnivores	22	409 ± 146^{B}	326 ± 12.0^{B}	1.25 ± 0.44^{BC}
Piscivores	15	272 ± 107^{B}	345 ± 7.1^{AB}	0.79 ± 0.31^{BC}
Carnivores	15	60 ± 40^{C}	341 ± 14.3^{AB}	0.18 ± 0.12^{C}

[a] Within columns, values not sharing the same letter are significantly different

The relationship between Sr and Ca concentrations in avian eggshells is shown in Fig. 2. Sr and Sr/Ca ratios were highly significantly correlated ($R^2 = 0.99$), but Sr and Ca were not ($R^2 = 0.006$) indicating that the ratio was highly dependent on the Sr concentration. Eggshell thickness was positively correlated with Ca ($R^2 = 0.37$), but poorly correlated with Sr ($R^2 = 0.03$). Birds from Roosevelt Lake showed significantly lower levels of Ca than most of the birds from other regions; thus, a polynomial quadratic equation provided the best fit for the relationship between eggshell thickness and Ca concentrations ($R^2 = 0.50$, $\text{Thickness} = 2.13 - 0.02\text{Ca} - 3.07 * 10^{-5}\text{Ca}^2$; Fig. 3). Additionally, there was a significant positive relationship ($R^2 = 0.58$, $P < 0.0001$; Fig. 4) between Sr/Ca ratios in surface water collected from regions close to those from where the eggshells were collected and mean Sr/Ca ratios in eggshells from 12 locations in California, Arizona, Texas, Idaho, Kansas, and Michigan. The following predictive equation was obtained: $(\text{Sr/Ca})_{\text{eggshell}} = 0.94\ (\text{Sr/Ca})_{\text{water}} - 0.85$, suggesting that Sr/Ca ratios in bird eggshells can be predicted from Sr/Ca ratios in surface waters from regions near a birds' breeding ground.

Discussion

The results from this study suggest that the accumulation of Sr and Ca in avian eggshells varies with region (as reflected by Sr/ca ratios in water), species, and foraging guild. These results are in agreement with other studies which indicate that the accumulation of Sr and Ca in biota depends on the availability of these elements in the environment and the biota's position in the food chain (Elias et al. 1982; Blum et al. 2000). Overall, our results indicate that regional

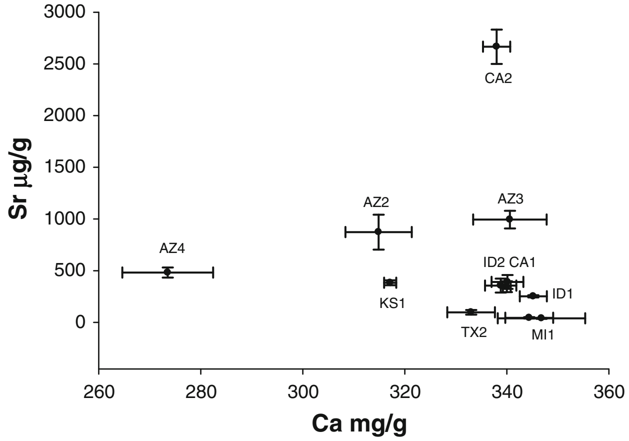

Fig. 2 Relationship of the distribution of Sr and Ca by region (mean ± SE). *AZ2* Lower San Pedro River AZ, *AZ3* Camp Verde AZ, *AZ4* Roosevelt Lake AZ, *CA1* Edwards Air Force Base CA, *CA2* Volta Wildlife Area CA, *ID1* Deer Flat NWR ID, *ID2* Bear Lake NWR ID, *KS1* Konza Prairie Nature Preserve KS, *MI1* Detroit MI, *MI2* Kalamazoo River MI, *TX1* Bryan TX, *TX2* Lower Rio Grande Valley TX

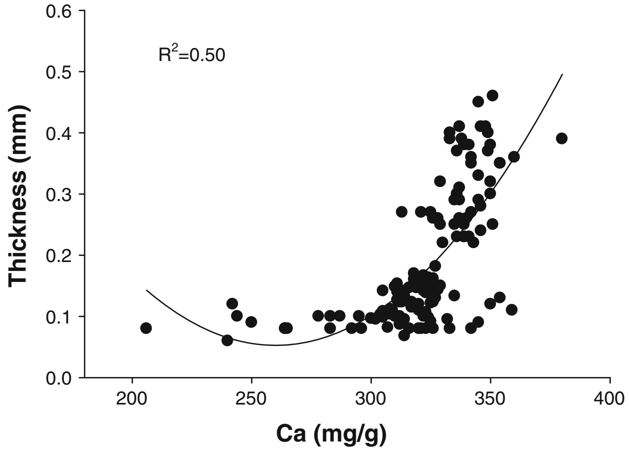

Fig. 3 Relationship between Ca concentration and eggshell thickness: Thickness = $2.13 - 0.02\mathrm{Ca} - 3.07 * 10^{-5}\mathrm{Ca}^2$. Points represent individual values from all the eggshells analyzed

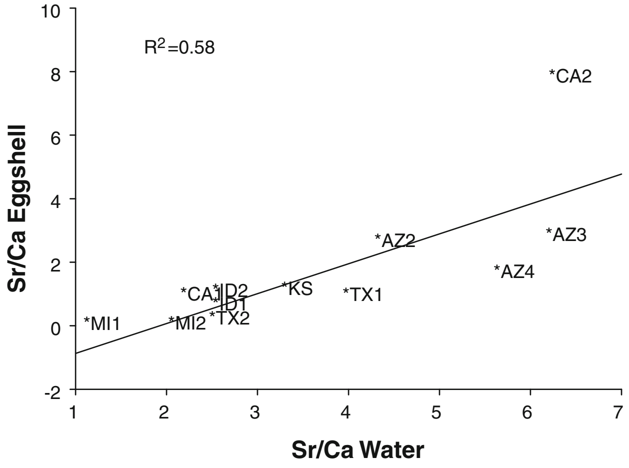

Fig. 4 Relationship between Sr/Ca in water and Sr/Ca in eggshells: $(\mathrm{Sr/Ca})_{\mathrm{eggshell}} = 0.94\ (\mathrm{Sr/Ca})_{\mathrm{water}} - 0.85$. Mean Sr/Ca values for surface water were taken from locations proximate to eggshell collection sites, as reported in Sillen and Kavanagh (1982). The water data correspond to a much earlier period (1960s) than that of the eggshell collections (1985–2007). Mean Sr/Ca values for eggshells were obtained by region, regardless of species. Acronyms represent the same regions as in Fig. 2

Sr/Ca ratios in surface water which are strongly associated with location are the best determinants of Sr and Sr/Ca ratios in birds. However, because of the biopurification of Ca or discrimination against Sr (i.e., Ca becomes more purified as it moves up in the food chain; Elias et al. 1982), the species' food habits are highly influential in determining the total amount of Sr, Ca, and Sr/Ca ratios accumulated in the eggshell. Within a given region, herbivores or animals feeding on plants, should be expected to have the highest Sr/Ca ratios; and carnivores, or species at the top of the food chain, should have the lowest (Elias et al. 1982). We observed significant differences in Sr concentrations and in Sr/Ca ratios among the six different foraging guilds; however, the highest concentrations were observed in invertivores, not in herbivores as the hypothesis predicts (Elias et al. 1982; Sillen and Kavanagh 1982). The invertivores were from Bear Lake National Wildlife Refuge, Idaho, and the Volta wildlife area near Los Banos, California. The lowest Sr and Sr/Ca ratios were observed in eggshells of three carnivore species from Michigan and Texas.

The significant differences observed in Sr, Ca, and Sr/Ca ratios among species, regions, and foraging guilds in this study should be examined carefully because it was not possible to analyze the same species from all locations, and seven locations were represented by a different species. Because of such variability, it would be difficult to determine if the differences observed are representative of a given species, a region, or both. However, the results of the analysis of foraging guilds with regional Sr/Ca ratios in water as a covariate provided further support that the region and position in the food chain are probably the two most important factors determining Sr concentrations and Sr/Ca ratios in avian eggshells. Additionally, there is enough information in the literature to support the assertion that Sr/Ca ratios in avian species are most likely a reflection of the values observed in soils and ground and surface water in the region (Sillen and Kavanagh 1982). Accordingly, the amount of Sr and Ca entering the food chain via plants is primarily a function of the amount of Sr and Ca in groundwater. Alexander et al. (1954) reported that the highest Sr concentrations in water were from regions with a large proportion of soft lime carbonate. Among the factors contributing to high Sr concentrations include, highly soluble parent material, high rates of evaporation, high salinity, and low annual rainfall (Sillen and Kavanagh 1982). The highest concentrations of Sr have been reported in the southwest, including Arizona, New Mexico, western Oklahoma, and northern Texas and the lowest concentrations in the Pacific northwest, southeastern United States, upper Great Lakes, and most of the east coast (Sillen and Kavanagh 1982).

Based on the assumption that the Sr/Ca ratios of rivers or drainage systems remain constant over a significant range and tend not to change much over time, we used Sr/Ca ratios of surface waters measured in nearby regions in the 1960s to correlate with Sr/Ca ratios in eggshells. We detected a highly significant linear relationship between Sr/Ca values in eggshells and those in surface water from nearby regions (Fig. 4). Thus, birds from regions with high Sr/Ca ratios in water also had high Sr concentrations and high Sr/Ca ratios in eggshells. The lowest Sr concentrations and Sr/Ca ratios were observed in birds from Michigan, a region also with reported low Sr/Ca ratios in water. However, the birds from Michigan also were species at the top of the food chain which are also expected to have lower Sr/Ca ratios than other species.

Our results provide additional evidence for the accumulation of Sr in the avian eggshell. The potential biological significance of elevated concentrations of Sr in wild bird eggshells, however, remains to be determined. The Sr concentrations measured in black-necked stilts (mean = 2,666 ± 372 μg/g dw) from California are among the highest ever reported in avian eggshells. These values are 2.3 to 2.7 times greater than the second and third highest concentrations observed in yellow warblers and brown-headed cowbirds from Lower San Pedro River and Camp Verde in Arizona. There are several studies that point out that Sr could interfere with embryo development and hatching success (Mraz et al. 1967; Elaroussi and Deluca 1994; Neufeld and Boskey 1994). Sr apparently interferes with the ability of the female to form an egg, either through damage to the egg or damage to the health of the female (Quintana et al. 1980). Concentrations of Sr as high as 176 μg/g dw were reported in unhatched eggs of California clapper rails from the San Francisco Bay area (Schwarzbach et al. 2006). Clapper rail embryo deaths occurred in the middle or late stages of incubation, a stage at which the concentration of Sr in the egg contents had increased (Schwarzbach et al. 2006). The dead clapper rail embryos exhibited polydactylus and stunted toe deformations. These results suggest that Sr concentration may increase in the embryo as a result of absorption from the eggshell. If this is the case, then elevated concentrations of Sr in the eggshell suggest potential transfer to the embryo with potential for increased deleterious effects. Mora et al. (2007) estimated that Sr eggshell/egg ratios varied between 6 and 93 for insectivorous (mostly) birds from Arizona. Hopkins et al. (2006) reported that embryonic development of the amphibian *Gastrophryne carolinensis* was impaired in sites with elevated concentrations of selenium and Sr in eggs. Abnormalities such as craniofacial and axial malformations were reported in hatchlings from the contaminated site and many individuals also showed impaired swimming behavior (Hopkins et al. 2006). However, Sr concentrations in eggs were not significantly related with any of the developmental parameters.

As indicated previously, most contaminant studies on avian eggs focus on egg contents and do not consider the eggshell as an important compartment. Our studies suggest that in regions of high Sr concentrations in rock or soil, it would be recommended to also measure Sr and Ca concentrations in the eggshell and correlate with various reproductive parameters. This information would help determine whether Sr could be a significant factor in affecting eggshell thickness and reproduction of avian species.

Acknowledgments We greatly appreciate the kindness and contribution of various individuals, including R. Hothem, D. Best, C. Thomas, and the Peregrine Fund Inc, who provided some of eggshells for the Sr analysis. R. Taylor and M. Woodin and two anonymous reviewers provided useful comments which improved the manuscript. M. Speed and D. Jann assisted with part of the data analysis. M. Blundell, J. Hite, B. Van Korff assisted with eggshell collection at Konza Prairie.

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