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Heavy metals and metalloids in egg contents and eggshells of passerine birds from Arizona

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"Capsule": *High concentrations of Sr in eggshells may be associated with lower hatching success of some passerine birds.*

Abstract

Concentrations of inorganic elements were determined in eggs of passerine birds including the endangered southwestern willow flycatcher (*Empidonax traillii extimus*) from four regions in Arizona. The main aim of the study was to determine the distribution of metals in egg contents and eggshells, with emphasis on the deposition of Sr in eggshells. Seventy eggs of 11 passerine species were collected at four nesting locations during 2000. Aluminum, Ba, Cr, Cu, Mn, Se, Sr, and Zn, were detected primarily in egg contents of all bird species. Arsenic, Ni, Pb, and V were detected primarily in eggshells. A proportion of most inorganic elements accumulated in the eggshell. Concentrations of Ba, Cu, Mn, Se, Sr, and Zn in egg contents and As, Ba, Cu, and V in eggshells of yellow-breasted chats (*Icteria virens*) were similar among locations. However, concentrations of Mn, Ni, Sr, and Zn in eggshells were significant different among locations. Except for Cu, Mn, Se, and Zn, concentrations of inorganic elements were 2–35 times greater in eggshells than in eggs. Most concentrations of metals and metalloids in eggs and eggshells of all the bird species were below levels known to affect reproduction or that have other deleterious effects. However, I found somewhat elevated concentrations of Sr in eggshells (highest mean = 1505 μg/g dw, $n = 3$) of yellow-breasted chats and willow flycatchers, and in egg contents of yellow warblers (*Dendroica petechia*) and song sparrows (*Melospiza melodia*). Whether current observed concentrations of Sr in eggshells are affecting nesting birds in Arizona remains to be determined. Strontium and other metals could be associated with lower hatching success in some areas. This study shows that a proportion of many inorganic elements accumulates in the eggshell and that the potential effects on the proper structure and functioning of the eggshell should not be ignored.
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Keywords: Arizona; Eggs; Eggshells; Metals; Strontium; Passerine birds

1. Introduction

Previously, we reported the results of a study that addressed the potential effects of environmental contaminants on the endangered southwestern willow flycatcher (*Empidonax traillii extimus*) in two regions of Arizona, the Lower San Pedro River and Roosevelt Lake (Mora et al., in press). The lower San Pedro River and Roosevelt Lake represent important riparian corridors for nesting of many passerine species. Some of these riparian habitats are affected by agricultural, mining, and water treatment activities. Environmental contaminants were of concern because several deformed flycatcher nestlings have been reported during the last few years in Arizona (Sogge and Paxton, 2000). Bill (shorter or offset mandible) and eye (missing eye) deformities were observed in about 1.4% of more than 1000 willow flycatchers banded from 1996 through 2000 in the southwestern United States (Sogge and Paxton, 2000). Some of the most common deformities observed in many bird species in the United States include crossed bills, small or missing eyes, and clubbed feet, but other type of deformities also occur (Ohlendorf et al., 1986, Gilbertson et al., 1991, Giesy et al., 1994).

In our previous study we documented DDE and PCBs at low concentrations in eggs and carcasses of passerine birds. These were the only organochlorine compounds quantified above detection limits. The concentrations of DDE and PCBs did not differ among locations or among species. Most concentrations of

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metals and metalloids in eggs and carcasses also were low and not of concern for negative effects such as deformities in birds. However, we found somewhat elevated concentrations of strontium (Sr) in eggs (Mora et al., in press). This raised concern for potential association with deformities in the willow flycatcher, but more documentation was needed. Due to the detection of low concentrations of organochlorine compounds but somewhat elevated concentrations of metals, particularly Sr, we decided to undertake a more comprehensive investigation of inorganic elements in bird eggs from additional riparian habitats where breeding populations of the willow flycatcher are found in Arizona. The objectives of this study were to evaluate concentrations of inorganic elements in eggs of willow flycatchers and related species nesting in selected regions in Arizona; and to determine concentrations of Sr and other metals sequestered in eggshells of laying females.

2. Materials and methods

2.1. Collection sites

Four known nesting locations of the willow flycatcher in Arizona were selected for collection of eggs. Two locations were the same as those sampled during 1999: the lower San Pedro River riparian zone and Roosevelt Lake in the Tonto National Forest; and the new sampling sites were located at Alamo Lake State Park and Camp Verde (Fig. 1).

2.2. Sample collection

Seventy eggs were collected during June–July 2000 at the four locations (Table 1). Some eggs, particularly non-viable willow flycatcher eggs, were salvaged by Arizona Game and Fish and US Geological Survey biologists. The majority of eggs were obtained from yellow-breasted chat (*Icteria virens*), Bell's vireo (*Vireo bellii*), yellow warbler (*Dendroica petechia)*, willow flycatcher, and brown-headed cowbird (*Molothrus ater*). Additionally, one or two eggs of common yellowthroat (*Geothlypis trichas)*, song sparrow (*Melospiza melodia*), black-throated gray warbler (*Dendroica nigrescens*), summer tanager (*Piranga rubra*), vermillion flycatcher (*pyrocephalus rubinus*), and lesser goldfinch (*Carduelis psaltria*) were obtained. Eggs were collected haphazardly, as nests were located and nesting species were identified; thus, the condition of the eggs varied from freshly laid, to nearly hatched, and addled eggs. Upon collection from the nest, some eggs were placed directly in egg cartons and others were wrapped in aluminum foil and placed in chemically cleaned glass jars. A few

Fig. 1. Sampling locations in Arizona (1) Lower San Pedro River, (2) Roosevelt Lake, (3) Camp Verde, (4) Alamo Lake.

Table 1
Mean metal and metalloid concentrations ± S.D. (μg/g dw) in eggs and eggshells of birds from Arizona, 2000

Class	Species[a]	Location[d]	N	% Moisture	As	Ba	Cu	Mn	Ni	Se	Sr	Pb	V	Zn
Egg	YBCH	San Pedro	8	84.6 ± 2.4	< 0.5	1.8 ± 1.5	3.4 ± 0.9	1.8 ± 0.6	0.6[b]	2.9 ± 0.6	24.6 ± 13.8	< 0.5	< 0.5	51 ± 10.6
						A	A	A		A	A			A
		Roosevelt	6	80 ± 9.6	< 0.5	2.5 ± 1.4	3.5 ± 1.0	3.3 ± 1.9	0.9[b]	2.8 ± 1.0	20.9 ± 10.2	< 0.5	0.6 ± 0.01	58.1 ± 12.5
						A	A	A		A	A			A
		Alamo	4	76.4 ± 12.7	< 0.5	1.9 ± 1.4	2.3 ± 0.7	4.1 ± 1.5	< 0.5	2.7 ± 0.9	17.7 ± 12.7	< 0.5	< 0.5	51 ± 20
						A	A	A		A	A			A
		C Verde	3	78.9 ± 2.1	< 0.5	5.3 ± 3.1	3.0 ± 0.7	1.7 ± 0.5	1.1 ± 0.5	4.0 ± 0.5	36.5 ± 10	< 0.5	< 0.5	46.4 ± 10.1
						A	A	A		A	A			A
	YWAR	San Pedro	2	90.2 ± 0.8	< 0.5	2.9 ± 0.7	3.1 ± 0.3	3.5 ± 0.01	< 0.5	3.8 ± 0.4	41.9 ± 16.3	1.8[b]	1.5[b]	68.1 ± 5.2
		Roosevelt	4	69[b]	1.3[b]	14.3 ± 9.8	4.1 ± 1.8	3.5 ± 1.2	1.5[b]	1.8 ± 1.1	224 ± 173	< 0.5	2.6[b]	59.4 ± 31
	BEVI	San Pedro	4	88.5 ± 1.7	< 0.5	2.2 ± 1.4	2.2 ± 0.3	1.5 ± 0.7	< 0.5	2.5 ± 0.9	22.5 ± 12	< 0.5	0.8[b]	46.2 ± 7.7
		Roosevelt	6	81.1 ± 2.6	< 0.5	3.2 ± 3.0	8.9 ± 17	12.5 ± 20.6	< 0.5	1.9 ± 1.0	13.0 ± 11.7	< 0.5	2.0[b]	79.8 ± 53.3
		Alamo	1	78.3	< 0.5	2.3	2.1	2.1	< 0.5	1.4	13.8	< 0.5	< 0.5	40.3
	WIFL	San Pedro	5	33.3[b]	< 0.5	0.4 ± 0.2	3.2 ± 0.8	2.1 ± 0.2	0.7 ± 0.3	3.1 ± 0.4	50.2 ± 51.9	< 0.5	< 0.5	42.3 ± 7.2
		Roosevelt	2	ND[c]	< 0.5	< 0.5	1.9 ± 0.5	0.8 ± 0.5	< 0.5	3.9 ± 0.1	3.2 ± 1.8	< 0.5	1.0[b]	31.3 ± 19.8
		Alamo	1	76.0	< 0.5	0.24	2.3	3.9	< 0.5	3.3	5.2	< 0.5	< 0.5	50
	COYE	San Pedro	1	78.6	< 0.5	14.1	3.9	1.9	< 0.5	2.5	153.7	< 0.5	< 0.5	62.5
		Roosevelt	1	ND	< 0.5	1.5	16.8	0.8	< 0.5	7.4	7.0	< 0.5	< 0.5	57.4
	BTYW	San Pedro	1	ND	< 0.5	1.9	2.7	0.6	0.6	3.8	5.0	< 0.5	< 0.5	30.9
	SUTA	San Pedro	1	ND	< 0.5	1.3	2.6	1.9	< 0.5	2.4	9.0	< 0.5	< 0.5	33.8
	VEFL	Roosevelt	5	82 ± 3.5	< 0.5	2.9 ± 1.7	2.8 ± 1.4	1.8 ± 0.8	< 0.5	3.2 ± 1.2	31.0 ± 21.0	< 0.5	1.8[b]	48.8 ± 24.3
	SOSP	San Pedro	2	ND	< 0.5	9.2 ± 11.6	2.4 ± 0.9	0.9 ± 0.4	1.1[b]	3.0 ± 0.6	189 ± 259	< 0.5	1.3[b]	35.4 ± 26
		Alamo	1	ND	< 0.5	3.2	3.0	3.4	< 0.5	2.7	15.8	< 0.5	< 0.5	34.5
		C Verde	1	73.0	< 0.5	11.6	1.7	2.6	0.7	2.6	16.4	< 0.5	< 0.5	79.8
	BHCO	San Pedro	2	ND	< 0.5	5.3 ± 1.3	3.0 ± 0.1	1.1 ± 0.1	< 0.5	2.1 ± 0.7	32.2 ± 13.5	< 0.5	< 0.5	34.3 ± 1.0
		Roosevelt	2	84.8 ± 2.3	< 0.5	2.5 ± 1.0	3.1 ± 0.6	1.4 ± 0.6	< 0.5	2.1 ± 1.1	22.5 ± 15	< 0.5	< 0.5	43.6 ± 12.5
		C Verde	6	80.6[b]	< 0.5	8.3 ± 2.3	3.0 ± 0.8	1.4 ± 0.7	2.5 ± 2.9	2.7 ± 0.4	46.4 ± 44.8	0.7[b]	1.1[b]	48.9 ± 12
	LEGO	C Verde	1	67.7	< 0.5	1.8	1.6	3.7	0.9	2.1	14.8	0.7	< 0.5	72.6
Shell	YBCH	San Pedro	8	ND	1.9 ± 0.3	26.4 ± 13	2.5 ± 1.8	1.4 ± 0.7	2.8 ± 1.9	< 0.5	913 ± 330	1.05[b]	5.8 ± 0.9	5.8 ± 4.6
					A	A	A	A	A		BC		A	AB
		Roosevelt	6	ND	2.4 ± 0.5	28.1 ± 13.2	7.4 ± 13.6	4.1 ± 2.2	2.6 ± 1.0	0.9 ± 0.1	433 ± 80	1.5 ± 0.6	4.5 ± 2.0	14.9 ± 14.5
					A	A	A	B	A		A		A	AB
		Alamo	4	ND	2.2 ± 0.2	27.9 ± 21.2	6.9 ± 5.6	4.2 ± 1.5	9.2 ± 5.4	< 0.5	734 ± 112	< 0.5	5.2 ± 0.2	3.3 ± 1.5
					A	A	A	B	B		AB		A	A
		C Verde	3	ND	1.8 ± 0.2	73.8 ± 29.7	12.5 ± 2.6	3.0 ± 0.6	3.6 ± 1.8	0.8 ± 0.2	1505 ± 425	< 0.5	5.0 ± 0.4	16.7 ± 9.6
					A	A	A	AB	AB		C		A	B
	WIFL	San Pedro	2	ND	1.2 ± 0.2	4.3 ± 3.4	3.1 ± 0.6	3.8 ± 1.1	1.4 ± 0.1	< 0.5	459 ± 89	< 0.5	7.8 ± 0.2	43.2 ± 5.8
		Roosevelt	2	ND	1.4 ± 0.3	2.9 ± 2.5	1.6 ± 0.2	3.1 ± 0.5	8.4 ± 5.4	1.8 ± 2.3	188 ± 48.5	1.5 ± 0.1	2.1 ± 0.4	43.3 ± 15.8
		Alamo	1	ND	1.1	3.4	5.7	6.1	12.8	0.95	201.3	< 0.5	4.2	57.9

[a] Yellow-breasted chat (YBCH), yellow warbler (YWAR), Bell's vireo (BEVI), willow flycatcher (WIFL), common yellowthroat (COYE), black-throated gray warbler (BTYW), summer tanager (SUTA), vermilion flycatcher (VEFL), song sparrow (SOSP), brown-headed cowbird (BHCO), lesser goldfinch (LEGO).
[b] Quantified above detection limits in one sample.
[c] ND, not determined.
[d] Means among locations for a given element sharing the same letter are not significantly different.

eggs were broken during handling, thus they were placed directly in chemically cleaned glass jars. All eggs were stored in the refrigerator within two hours of collection until further processing. In the laboratory, intact eggs were washed with distilled water and were allowed to dry at room temperature. Egg contents were obtained by cutting the eggshell at the equator with a surgical blade previously rinsed with acetone. Eggshells were rinsed with distilled water and then with acetone.

One grab sediment sample was collected from Alamo Lake, lower San Pedro River, and Roosevelt Lake. The sediments were collected in a chemically cleaned glass jar and were kept on ice until taken to the laboratory and stored at −20 °C until analyzed. The sediments were analyzed for the same inorganic elements as the eggs.

2.3. Chemical analysis

Egg contents of all species and eggshells of yellow-breasted chats and willow flycatchers were analyzed for metals and metalloids at the Geochemical and Environmental Research Group, Texas A&M University. The elements analyzed were Aluminum (Al), arsenic (As), barium (Ba), boron (B), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), lead (Pb), selenium (Se), strontium (Sr), vanadium (V), and zinc (Zn). Approximately 0.2–0.5 g of sample were digested in screw-cap Teflon bombs with concentrated high purity nitric acid. Bombs were heated from 2 to 8 h and opened three times to release $C0_2$ buildup. Most elements in the digestate were analyzed with a Perkin-

Elmer, model ELAN 5000, inductively coupled plasma spectrometer. Arsenic, Se, Pb, and Cd were analyzed with a Varian VGA-76 hydride generation accessory mounted to an atomic absorption spectrophotometer, AA Perkin-Elmer, model 3030. Due to the small amount of egg content, Hg could not be quantified in many eggs. However, when enough sample was available, mercury was analyzed by the standard cold vapor atomic absorption method. The lowest detection limits for Al, As, B, Ba, Be, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sr, V, and Zn were 0.5, 0.5, 2, 1, 0.1, 0.1, 0.5, 0.5, 5, 0.2, 1, 2, 0.5, 0.5, 0.5, 0.5, 0.5, and 1 μg/g dw, respectively. The method detection limits were calculated by a standard procedure which is based on the analysis of seven samples of the matrix with the analyte (Glaser et al., 1981). Percent recoveries of spiked samples and certified reference materials were above 90% in most cases. Mean relative percent differences between duplicates were $<10\%$. Percent moisture was obtained by drying out approximately 1 g of sample at about 75 °C for 24 h or until constant weight. Percent moisture was determined only in about 90% of the eggs due to sample limitations.

2.4. Statistical analysis

The metal contaminant data were not normally distributed, therefore, comparisons of mean concentrations in eggs and eggshells, by species and among sites, were performed with the GLM procedure on ranked data (SAS Institute, 1987). The Tukey multiple comparisons procedure was used to determine which means were significantly different. Differences in concentrations of inorganic elements among locations were determined only with data from yellow-breasted chats. Differences in concentrations between eggs and eggshells of yellow-breasted chats were determined by paired *t*-tests. Differences between chats and flycatchers were determined by two sample comparison procedures. The level of significance was set at 0.05.

3. Results

Inorganic elements detected in egg contents of all bird species included primarily Al, Ba, Cr, Cu, Mn, Se, Sr, and Zn. More metals were detected in eggshells and included As, Ni, Pb, and V, in addition to the elements detected in egg contents (Table 1). Lead was predominantly found in eggshells of yellow-breasted chats and willow flycatchers from Roosevelt Lake and one eggshell from the lower San Pedro River. However, Pb also was found above detection limits in two eggs from Camp Verde and one egg from the San Pedro River (Table 1). Arsenic was below detection limits in egg contents, except for one sample from Roosevelt Lake with 1.3 μg/g dw; however, it was present in all eggshells of yellow-breasted chats and willow flycatchers (Table 1). Vanadium also was found above detection limits in three egg contents in addition to the eggshells. Selenium was measured above detection limits in all egg contents and in 31% (8 of 26) eggshells. Concentrations of B, Be, Cd, and Hg were below detection limits and are not included in Table 1. Also, because of possible contamination with aluminum foil in a few broken eggs, concentrations of Al and Cr are not reported in this study.

3.1. Comparisons among locations

Comparisons among locations were possible with concentrations of inorganic elements in yellow-breasted chats only (first four rows for egg contents and first four rows for eggshells in Table 1). Species comparisons within or among locations were not possible because of small sample sizes. Concentrations of all elements in egg contents of yellow-breasted chats were similar among locations (Table 1). However, concentrations of Mn, Ni, Sr, and Zn in eggshells differed significantly at some locations. Concentrations of Mn were significantly higher ($P<0.05$) in eggshells from Alamo and Roosevelt lakes than in those from the lower San Pedro River, but were similar to those from Camp Verde. Nickel concentrations were significantly higher in eggshells of birds from Alamo Lake than in those from Roosevelt Lake and lower San Pedro River ($P<0.05$), but were similar to those from Camp Verde. Concentrations of Sr were significantly higher in eggshells of birds from Camp Verde than in those from Alamo and Roosevelt lakes ($P<0.05$), but were similar to those from the lower San Pedro River. Concentrations of Zn were significantly greater ($P<0.01$) in yellow-breasted chats from Camp Verde than in those from Alamo Lake, but were similar to those from the lower San Pedro River and Roosevelt Lake.

3.2. Concentrations in egg contents vs eggshells

A proportion of all the inorganic elements analyzed in this study was concentrated in the eggshell of yellow-breasted chats and willow flycatchers (Table 2). Except for Se, Cu, Mn, and Zn, concentrations of other inorganic elements were significantly different ($P<0.05$) and were 2–35 times greater in eggshells than in eggs (Table 2). Barium, Ni, Sr, and V were, on average (both species), 10–20 times more concentrated in the eggshell than in egg contents. In contrast, concentrations of Se and Zn were approximately six times greater in egg contents than in eggshells of yellow-breasted chats. Selenium also was nearly three times greater in eggs than in eggshells of willow flycatchers, whereas Zn was similar.

Table 2
Mean concentrations (μg/g dw±S.D.) and eggshell/egg ratios of inorganic elements in yellow-breasted chats ($N=21$) and willow flycatchers ($N=5$) from Arizona

Element	Species					
	Yellow-breasted chat			Willow flycatcher		
	Egg	Eggshell	Eggshell/Egg	Egg	Eggshell	Eggshell/Egg
As	<0.5[a]	2.1±0.4	8.4	<0.5	1.3±0.2	5.2
Ba	2.5±2	33.9±23	13.6	0.5±0.1	3.6±2.2	7.2
Cu	3.2±0.9	6.2±8	1.9	2.5±0.9	3.0±1.7	1.2
Mn	2.7±1.5	2.9±1.9	1.1	1.9±1.3	3.9±1.4	2.1
Ni	<0.5	4.1±3.6	16.4	<0.5	6.5±5.7	26
Pb	<0.5[b]	0.6±0.7	2.4	<0.5	0.9±0.6	3.6
Se	3.0±0.8	0.5[c]±0.3	0.17	3.4±0.4	1.2±0.7	0.35
Sr	23.9±12.7	826±424	34.6	35.1±58	299±155	8.5
V	<0.5[d]	5.2±1.3	20.8	<0.5	4.8±2.9	19.2
Zn	52.4±12.8	9.5±10	0.18	38.5±12.4	46.2±10.7	1.2

[a] As was below detection limits in egg contents, except for 1 sample with 1.3 μg/g dw. Used 0.5 the DL to calculate ratio.
[b] Pb was not detected in egg contents of YBHCs or WIFLs. Used 0.5 DL to calculate ratio.
[c] Se was detected above detection limits in 31% (8 of 26) of the eggshells.
[d] Vanadium was found above detection limits in three YBCH egg contents. Used 0.5 DL to calculate ratio.

3.3. Yellow-breasted chats vs willow flycatchers

Concentrations of most inorganic elements in egg contents were similar between the two species except for Ba which was significantly higher ($P<0.001$) in yellow-breasted chats than in willow flycatchers. Concentrations of most inorganic elements in eggshells also were not significantly different between chats and flycatchers, except for As, Sr, and Zn. Arsenic and Sr were significantly higher ($P<0.001$) in eggshells of yellow breasted-chats than in willow flycatchers. Zinc was significantly higher ($P<0.01$) in eggshells of willow flycatchers than in chats. The eggshell/egg ratios were similar between yellow-breasted chats and willow flycatchers ($P > 0.05$); however, Zn seemed proportionally less concentrated in eggshells of yellow-breasted chats than in willow flycatchers (Table 2).

3.4. Concentrations in sediments

Concentrations of inorganic elements in sediments collected from three locations are shown in table 3. Concentrations of Sr were somewhat elevated. Concentrations of elements of concern such as Hg and Se were relatively low, except for Se in one sample from Alamo, which seemed somewhat high.

4. Discussion

4.1. Relevancy of concentrations and potential effects

Most concentrations of metals and metalloids in eggs and eggshells of all the bird species collected at the four locations in Arizona during 2000 were at levels that have not been associated with lowered reproduction or other detrimental effects on birds (Eisler 1985, 1986, 1987, 1988, 1998; Whitworth et al., 1991, Heinz, 1979, McIlveen and Negusanti, 1994). However, there were somewhat elevated concentrations of Sr in eggshells of yellow-breasted chats and willow flycatchers, and in egg contents of yellow warblers and song sparrows. Such high concentrations may be of some concern for potential detrimental effects on passerine birds nesting on some riparian habitats in Arizona, but field and laboratory documentation of such effects is needed.

Although no significant differences in egg contents of yellow-breasted chats were detected among locations for all elements, maximum mean concentrations of Cu, Mn, and Zn, were found in egg contents of Bell's vireos from Roosevelt Lake. Mean Se levels in eggs of all bird species sampled in Arizona were 2–12 times lower than those that have been associated with deformities in aquatic birds. Skorupa and Ohlendorf (1991) suggested

Table 3
Concentrations of inorganic elements (μg/g dw) in sediment from three locations in Arizona, 2000

Location	Al	As	B	Ba	Be	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Se	Sr	V	Zn
San Pedro	10,078	5	9	101	0.8	0.25	17	48	13,923	0.06	614	11	17	0.35	163	21	43
Roosevelt	11,073	6	7	87	1.0	0.34	29	13	14,243	0.03	355	16	9	0.28	44	26	35
Alamo	33,306	10	29	204	2.3	0.39	53	68	37,743	0.04	836	45	21	1.7	230	49	92

that some teratogenic effects in aquatic birds are observed at a mean egg Se threshold range of 13–24 μg/g dw.

One significant finding of this study was the detection of elevated levels of Sr in eggshells of yellow-breasted chats and in eggs of yellow warblers and song sparrows. The concentrations of Sr in eggshells of willow flycatchers were similar to those reported in eggshells of the black-throated blue warbler (*Dendroica caerulescens*) from the northeastern United States (Blum et al., 2000). However, concentrations of Sr in eggshells of yellow-breasted chats were three times greater than those in willow flycatchers from Arizona and warblers from the northeast.

The distribution of Sr in the environment is associated with potassium- or calcium-rich rocks and some areas in Arizona have been reported with anomalous concentrations of Sr in stream sediments (Theobald and Barton, 1988). Inputs of Ca and Sr may be derived primarily from weathering of underlying soil and rock and atmospheric deposition, since this has been considered quite significant in certain areas of the United States (Vitousek et al., 1999). Thus, high concentrations of Sr in eggshells and eggs of passerine birds nesting in some regions of Arizona are probably a result of high deposits in the environment. Insects and invertebrates are the main source of Sr in the diet of passerine birds in Arizona. Earthworms were considered important in recycling Sr within soils and transfer of Sr through terrestrial food chains (Morgan et al., 2001). Strontium concentrations in insects collected in 1999 varied from 8 to 117 μg/g dw (Mora et al., in press) and in sediments of three locations ranged from 44 to 230 μg/g dw.

The presence of high concentrations of Sr in eggshells of yellow-breasted chats and in willow flycatchers to some extent, as well as in egg contents of yellow warblers and song sparrows deserves some attention. Strontium is strongly associated with calcium metabolism, thus, higher Ca requirements for the female during egg production result in increased Ca absorption in the gut (Kottferova et al., 2001), as well as increased absorption of Sr. Concentrations of Sr were elevated relative to Ca in eggshells of black-throated blue warblers in the northeastern United States (Blum et al., 2000). There is not enough information in the literature to establish whether these concentrations of stable Sr are deleterious to birds. Strontium at high concentrations was embryotoxic but not teratogenic to chickens (Ridgway and Karnofsky, 1952). Egg production declined in laying leghorn hens after feeding on diets containing 2 and 4% Sr (Mraz et al., 1967). At such concentrations, Sr reduced eggshell strength and increased the number of cracked eggs. Consequently, higher embryonic mortality was observed during the third week of incubation. Mraz et al. (1967) hypothesized that the calcite lattice of the outer eggshell was distorted by the presence of higher concentrations of Sr. Concentrations of Sr of 11.3 μg/g dw in pipping black-crowned night-herons (*Nycticorax nycticorax*) from Delaware Bay were associated with increased hepatic oxidative stress as measured by a two-fold elevation in hepatic oxidized glutathione concentration GssG (Rattner et al., 2000).

Whether current observed concentrations of Sr in eggshells are affecting nesting birds in Arizona remains to be determined. It is possible that the observed high levels of Sr in some species could contribute to increased egg breakage, reduced hatching success, and increased embryo mortality. Strontium interferes with normal metabolism of vitamin D by blocking the renal synthesis of 1,25-dihydroxyvitamin D (Moon, 1994). Vitamin D is essential for the utilization of eggshell Ca by the developing embryo since it facilitates the active transport of Ca by the chorioallantoic membrane. Vitamin D deficient embryos of Japanese quail (*Coturnix japonica*) were unable to hatch because they could not obtain Ca from the shell (Elaroussi and Deluca, 1994). Thus, if Sr interferes with vitamin D metabolism, high concentrations of Sr in the eggshell could result in decreased hatching success or insufficient transport of Ca for bone formation which could result in rickets (Neufeld and Boskey, 1994) or bill deformities. $Strontium^{2+}$ administered to rats on low Ca^{2+} diets decreased bone growth and produced endochondrial mineralization, suggesting that Sr inhibited the bone calcification process (Matsumoto, 1976).

Currently, it is not known if bill deformities in willow flycatchers are associated with high concentrations of Sr in eggshells. The rate of eye and bill deformities observed in flycatchers banded from 1996 to 2000 was relatively low (1.4%, Sogge and Paxton, 2000) and no information on deformities in other passerine birds nesting in the same areas is currently available. Surveys conducted by the Arizona Department of Game and Fish determined that the willow flycatcher had, on average, 80% hatching success during the period 1997–2000 (C. Paradzick, personal communication). Also, during the same period, the hatching success of the willow flycatcher at the Tonto Creek in the Roosevelt Lake area was only 72% (C. Paradzick, personal communication). The Tonto Creek is the same area where some of the highest concentrations of Cu, Zn, Sr, and Se were found in birds. Considering 80% as an average normal hatching success, then it is possible to hypothesize that the lower hatching success at the Tonto Creek area could be associated with high concentrations of Sr and other metals in birds nesting in this area.

4.2. Role of the avian eggshell in sequestering inorganic elements

There are not many studies that have reported concentrations of inorganic elements in eggshells. Dauwe et al. (1999) pointed out that some passerine species sequester non-essential heavy metals in the eggshell. Burger (1994) found that concentrations of Pb, Hg, Se, and Cr were significantly higher in egg contents than in eggshells and Cd and Mn were higher in shells than in eggs of Herring gulls (*Larus argentatus*). Hg and Se had the lowest concentrations in eggshells. Morera et al. (1997) found that concentrations of elements such as Zn, Cu, Mn, and Hg were 80–99% greater in egg contents than in eggshells of Audouin's gulls (*Larus audouinii*). Our results show that concentrations of As, Ba, Ni, Pb, Sr, and V were 2–35 times greater in eggshells than in egg contents of two passerine birds from Arizona. This study shows that a good number of inorganic elements are accumulated in the eggshell and that the potential effects of some of these elements on the proper structure and functioning of the eggshell should not be ignored. Considerable transport of eggshell solids and minerals into the embryo occurs during the latter half of incubation (Romanoff, 1967). Therefore, there is a potential for toxic minerals in the shell to become mobilized and perhaps affect later stage embryos, including critical strength of the embryo itself needed for hatching success.

5. Conclusions

Overall, most metals and metalloids analyzed in this study could not be implicated in the deformities observed in willow flycatchers in the Lower San Pedro River and other areas in Arizona; although the possible role of Sr deserves further field and laboratory work. Strontium and other metals may be contributing to the lower hatching success (C. Paradzick, personal communication) observed in birds from Tonto Creek, Roosevelt Lake. The eggs from surrogate bird species collected during 2000 were occupying the same habitat of the willow flycatcher in Arizona and were probably feeding on the same diet (most were insectivores) as the willow flycatcher (Drost et al., 1998; Yard, 1998). The metal residues in the few salvaged eggs from willow flycatchers were similar to those in the surrogate species. Willow flycatcher deformities may be due to other factors including contaminants that were not measured in this study. The willow flycatcher has been perhaps the most studied single species in Arizona over the last five years; thus, the incidence of deformities observed could be just a random event. The potential effects of elevated concentrations of Sr in yellow-breasted chat eggshells and in egg contents of yellow warblers and song sparrows could be further explored by documenting the degree of eggshell thickness and breakage associated with increased Sr levels in eggshells. Environmental contaminants such as metals, organochlorines, and other persistent chemicals would be expected to exert their toxic and teratogenic effects on the developing embryo or young rather than on adults, therefore, there is a need for more information on mortality rates and deformities in young to better assess exposure and effects of contaminants on the willow flycatcher.

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