Arch. Environ. Contam. Toxicol. 53, 103–109 (2007)
DOI: 10.1007/s00244-006-0139-7

ARCHIVES OF
Environmental
Contamination
and Toxicology

Selenium and Metal Concentrations in Waterbird Eggs and Chicks at Agassiz National Wildlife Refuge, Minnesota

Thomas W. Custer,[1] Christine M. Custer,[1] Bruce A. Eichhorst,[2] David Warburton[3]

[1] US Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, WI 54603, USA
[2] Biology Department, University of Nebraska at Kearney, Kearney, NE 68849, USA
[3] US Fish and Wildlife Service, 4101 E. 80th Street, Bloomington, MN 55425, USA

Received: 14 July 2006 / Accepted: 25 December 2006

Abstract. Exceptionally high cadmium (Cd) and chromium (Cr) concentrations were reported in eggs, feathers, or livers of selected waterbird species nesting at Agassiz National Wildlife Refuge (Agassiz) in 1994. Ten- to 15-day-old Franklin's gull (*Larus pipixcan*), black-crowned night-heron (*Nycticorax nycticorax*), and eared grebe (*Podiceps nigricollis*) chicks were collected in 1998, 1999, and 2001 at Agassiz and analyzed for selenium (Se) and metals including Cd and Cr. Freshly laid eggs were collected in 2001 from Franklin's gull, black-crowned night-heron, eared grebe, and pied-billed grebe (*Podilymbus podiceps*) nests at Agassiz. Based on a multivariate analysis, the pattern of Se and metal concentrations differed among species for eggs, chick feathers, and chick livers. Low Cd and Cr concentrations were measured in eggs, chick livers, and chick feathers of all four species. Mercury concentrations in black-crowned night-heron and eared grebe eggs collected from Agassiz in 2001 were lower than concentrations reported in 1994. Se and metal concentrations, including Cd and Cr, in waterbird eggs and chicks collected at Agassiz in 1998, 1999, and 2001 were not at toxic levels.

Key words: Metals—Cadmium—Chromium—Mercury—Selenium—Bird eggs—Feathers—Bird livers—Waterbirds

Exceptionally high concentrations of cadmium (Cd) and chromium (Cr) were reported in waterbirds at Agassiz National Wildlife Refuge (Agassiz) in northwestern Minnesota in 1994 (Burger and Gochfeld 1996a, 1996b, 1999). For example, mean Cd concentrations in eared grebe (*Podiceps nigricollis*) eggs from Agassiz in 1994 averaged 3.5 μg/g dry weight (Burger and Gochfeld 1996a; 0.74 μg/g wet weight, assuming 79% moisture). The median Cd concentration in eggs of raptors, seabirds, and other fish-eating birds from 32 studies was 0.015 μg/g wet weight (range: 0.002–0.600 μg/g wet weight; Burger 2002). Cadmium concentrations in feathers of young black-crowned night-heron (*Nycticorax nycticorax*) from Agassiz averaged 1.3 μg/g dry weight (Burger and Gochfeld 1996a); the median concentration of Cd in feathers based on 63 other studies was only 0.1 μg/g dry weight; the range was 0–24 μg/g dry weight (Burger 1996). Cr concentrations in Franklin's gull (*Larus pipixcan*) chicks collected at Agassiz in 1994 averaged 16.2 μg/g dry weight in livers (Burger and Gochfeld 1999; assuming 73% moisture) and 3.1 μg/g dry weight in eggs (Burger and Gochfeld 1996b). A Cr concentration greater than 4.0 μg/g dry weight in bird livers is presumptive evidence of Cr contamination (Eisler 2000). The median Cr concentration in eggs of raptors, seabirds, and other fish-eating birds from 21 studies (Burger 2002) was 0.8 μg/g dry weight (range: 0.04–3.9 μg/g dry weight; assuming 74.1% moisture).

Cadmium and Cr are toxic to birds in both laboratory and field studies. Toxic effects of Cd on birds include decreased egg production, kidney damage, testicular damage, and altered behavioral response (Furness 1996). Cr at high environmental concentrations is a mutagen, teratogen, and carcinogen (Eisler 2000). In one study, black duck (*Anas rubripes*) ducklings fed a diet high in Cr had altered growth patterns and reduced survival (Eisler 2000).

Because Agassiz is isolated from industry and large urban areas, the source of Cd and Cr contamination at Agassiz was puzzling but could have been related to the use of agricultural fertilizers. Cd is released into the environment by smelting, burning of coal and oils, and from using phosphate rock fertilizers (Furness 1996). A major source of Cr is from Cr alloy and metal-producing industries. Cr associated with phosphates used as fertilizers might also be an important source of Cr in soil and water (Eisler 2000).

Because of these high reported concentrations and the potential for adverse effects, this investigation was initiated in 1998 to reevaluate Se and metal contamination in Franklin's gulls, black-crowned night-herons, eared grebes, and pied-billed grebes (*Podilymbus podiceps*) nesting at Agassiz with emphasis on Cd and Cr. A concurrent study on tree swallows (*Tachycineta bicolor*; Custer *et al.* 2006) was initiated to determine whether metal exposure to birds was associated with specific drainages into Agassiz.

Correspondence to: Thomas W. Custer; *email:* tcuster@usgs.gov

Materials and Methods

Sample Collection

Five Franklin's gull, black-crowned night-heron, and eared grebe chicks 10- to 15-days of age were collected in 1998, 1999, and 2001 from Agassiz, Marshall County, Minnesota (48°21′N, 95°57′W). Black-crowned night-heron chicks were collected one per nest; Franklin's gull and eared grebe chicks were collected away from the nest. The chicks were euthanized by decapitation (1998 and 2001). Immediately after death, the liver was removed, weighed (±0.1 g), placed in a chemically clean jar, and frozen at −20°C until analysis.

In 1999, because feather samples were also collected, the birds were euthanized with carbon dioxide. Because of field conditions and time considerations, the carcasses were placed in individual sealed plastic bags and frozen at −20°C for later feather removal and dissection. These birds were later thawed and the feathers removed from each side of the body. Feathers from one side of body (randomly selected) were washed vigorously in deionized water alternated with acetone to remove loosely adherent external contamination (Burger and Gochfeld 1996a). This washing consisted of a rinse in deionized water followed by 15 min in an ultrasonic bath, one rinse in acetone followed by 15 min in an ultrasonic bath, and two more rinses in deionized water, each followed by an ultrasonic bath. Feathers from the other side of the body were not washed. Feather samples were placed into individual chemically clean jars and frozen at −20°C until analysis. Livers were removed, weighed (±0.1 g), placed in separate chemically clean jars, and frozen at −20°C until analysis.

In 2001, five fresh eggs of Franklin's gull, black-crowned night-heron, eared grebe, and pied-billed grebe were collected, one per nest, from Agassiz. An egg was considered fresh if it sank when placed in water and assumed a horizontal position (Hays and LeCroy 1971). Eggs were weighed (±0.01 g) and the length (±0.01 mm) and width (±0.01 mm) measured with a digital caliper. Eggs were opened, the contents emptied into a chemically clean jar, and the sample frozen at −20°C until analysis.

Chemical Analysis

Egg contents, chick livers, and chick feathers were analyzed for Se and metals by Research Triangle Institute (Research Triangle Park, NC, USA) using Environmental Protection Agency (EPA) procedures (EPA 1991). Samples were freeze-dried, weighed, and then homogenized in a blender. Subsamples of freeze-dried livers were digested in stages with heat and nitric–perchloric acid and then analyzed for Se and arsenic (As) by graphite furnace atomic absorption spectrophotometry, and for aluminum (Al), barium (Ba), beryllium (Be), boron (B), Cd, Cr, copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), strontium (Sr), vanadium (V), and zinc (Zn) by inductively coupled plasma–atomic emission spectrophotometry. Separate subsamples were digested by nitric acid reflux and analyzed for total Hg by cold vapor atomic absorption spectrophotometry. Nominal levels of detection (μg/g dry weight) in 1998 and 1999 (first number in parentheses following Se or metal designation) and 2001 (second number) were determined for the following metals and Se: Al (5.0, 1.0), As (0.5, 0.5), Ba (0.5, 1.0), B (2.0, 0.5), Be (0.1, 0.03), Cd (0.1, 0.1), Cr (0.5, 0.1), Cu (0.5, 0.1), Fe (10.0, 5.0), Hg (0.1 in 1998 and 0.05 in 1999 and 2001), Mg (10.0, 5.0), Mn (0.5, 0.5), Mo (0.5, 1.0), Ni (0.5, 0.1), Pb (1.0, 0.05), Se (0.5, 0.5), Sr (0.2, 0.2), V (0.5, 0.1), and Zn (1.0, 1.0). The number of spikes, duplicates, and blanks was 10% of the total number of samples analyzed. Concentrations were not adjusted for recovery, which averaged 106% ($n = 7$), 101% ($n = 10$), and 97% ($n = 11$) in 1998, 1999, and 2001, respectively, for 19 metals and Se. Concentrations are reported on a dry-weight basis. Approximate wet-weight concentrations can be calculated by using the mean percent moisture for fresh eggs of 78.7% for eared grebes, 73.1% for pied-billed grebes, 74.1% for Franklin's gulls, and 73.0% for black-crowned night-herons. Mean percent moisture for eared grebe, Franklin's gull, and black-crowned night-heron chick livers was 75.8%, 73.1%, and 74.8%, respectively. Mean percent moisture of unwashed feathers was 47.3% for eared grebes, 63.9% for Franklin's gulls, and 76.3% for black-crowned night-herons. We suspect that the high percentage of moisture in the feathers was due to condensation following thawing. It is possible that blood supply to the rapidly growing feathers could have contributed some moisture; however, dried blood on the feathers was not obvious during the plucking procedure. Percent moisture contents derived from this study were used when converting wet weight to dry weight for comparative purposes.

Statistical Procedures

Analysis of similarity (ANOSIM, PRIMER-E) was used to test for differences in the pattern of elements in eggs and nestling feathers among species; the pattern of elements in nestling livers was tested among years and species (Clarke and Warwick 2001). Analysis of similarity is a multivariate analogue of analysis of variance (ANOVA); it is built on a simple nonparametric permutation procedure and applied to the rank similarity matrix underlying the ordination of samples. The test statistic, R, might vary from +1 to −1. An R-value close to +1 indicates that there are very clear differences in patterns among the groups being tested. A value near zero means that the groups do not differ because the distribution of patterns is as similar among the groups as within the groups. Differences in patterns are evident when $R \geq 0.4$. There is some support for pattern differences when $R \geq 0.3$ and <0.4; no pattern is apparent when $R < 0.3$ (adapted from Clarke and Warwick 2001). Marginal effects of site and year were assessed using the equivalent of a two-way ANOVA without interaction (termed *two-way crossed analysis*). If an R-value of the main factor in a one-way or two-way ANOSIM test was <0.30, we did not present the R-values for the pairwise comparisons. Variables were included in the analyses if ≥50% of samples had detectable values; one-half the detection limit was assigned to samples below the detection limit. Se and metal concentrations were log-transformed prior to ANOSIM analysis.

Comparisons among species for individual contaminants were made using ANOVA when justified by the multivariate analysis. Based on visual inspection of residuals from models of log-transformed measures, models appeared to satisfy homogeneity of variance assumptions. Geometric means (antilog of mean log values) and 95% confidence intervals (CIs) are presented in the tables and text. Sample means were compared using a Bonferroni correction, and an overall α level of 0.05.

Results

Eggs

Aluminum, Cu, Fe, Hg, Mg, Mn, Se, Sr, and Zn were detected in all five Franklin's gull (FG, abbreviation used when in parentheses), black-crowned night-heron (NH), eared grebe (EG), and pied-billed grebe (PG) eggs collected at Agassiz in 2001. Ba was detected in all five Franklin's gull eggs and two black-crowned night-heron eggs. As (one detected in FG), B (one FG, one NH), Cr (one EG, one FG), Mo (one NH), and Ni (two EG, two FG) were detected in fewer than five eggs. Be, Cd, Pb, and V were not detected in eggs. Maximum Cr concentration was 0.3 μg/g dry weight in one Franklin's gull egg and 0.2 μg/g dry weight in one eared grebe egg. The maximum Hg concentration was 1.4 μg/g dry weight in one pied-billed grebe egg.

The pattern of Se and metal (Al, Cu, Fe, Hg, Mg, Mn, Sr, and Zn) concentrations differed among eggs of the four species (overall R among species = 0.56, Table 1). All species pairs differed except the comparison between eared grebes and Franklin's gulls ($R = 0.21$). Based on mean comparisons, differences in element concentrations in eggs, identified in the one-way ANOSIM (Table 1), were influenced by species differences associated with higher concentrations of Sr in Franklin's gulls; Cu, Mn, and Se in black-crowned night herons; Se in eared grebes; and Hg and Mn in pied-billed grebes (Table 2).

Feathers

Aluminum, Cu, Fe, Hg, Mg, Mn, Sr, and Zn were detected in feathers of all Franklin's gull, black-crowned night-heron, and eared grebe chicks ($n = 5$ per species) collected at Agassiz in 1999. Cd was detected in four eared grebe and three black-crowned night-heron samples. Se was detected in three eared grebe samples. Ba was detected in all five Franklin's gull samples, three eared grebe samples, and no black-crowned night-heron samples. Be (one detected in EG), Ni (one EG, 2 FG), and Pb (two EG) were detected in three or fewer samples. As, B, Cr, Mo, and V were not detected in any feather samples.

The pattern of Se and metal (Al, Cd, Cu, Fe, Hg, Mg, Mn, Sr, and Zn) concentrations in feathers differed among species (overall R among species equaled 0.94; Table 1); all species pairs differed. Based on mean comparisons, differences in Se and metal concentrations in feathers were influenced by higher concentrations of Al and Fe and lower concentrations of Hg in Franklin's gulls, higher concentrations of Mg and lower concentrations of Cu, Mn, and Zn in black-crowned night-herons, and higher concentrations of Cu and Mn and lower concentrations of Se in eared grebes (Table 3).

Livers

Copper, Fe, Mg, Mn, Se, and Zn were detected in all Franklin's gull, black-crowned night-heron, and eared grebe chick livers collected from Agassiz in 1998, 1999, and 2001 ($n = 5$ per species per year). Al (9 detected in FG, 8 NH, 8 EG), As (4 FG, 2 NH), B (15 FG, 6 NH, 13 EG), Cd (6 FG, 2 NH), Cr (5 FG, 4 NH, 5 EG), Hg (13 FG, 15 NH, 15 EG), Mo (15 FG, 1 NH, 11 EG), Ni (3 FG, 2 NH, 2 EG), Sr (10 FG, 6 NH, 9 EG), and V (1 FG) were detected in some but not in all livers. Ba, Be, and Pb were not detected in any liver sample. The maximum concentrations of Hg, Cr, and Cd were 1.16, 0.18, and 0.18 μg/g dry weight, respectively.

The pattern of Se and metal (Cu, Fe, Hg, Mg, Mn, and Zn) concentrations in waterbird livers differed among species and years (two-way ANOSIM; Table 4). Differences among species were evident (overall $R = 0.525$), as were all species comparisons ($R > 0.48$). There was also some support for differences among years ($R = 0.38$) and pairwise yearly comparisons ($R > 0.30$).

Table 1. Analysis of similarity for Se and metal patterns in eggs and in chick feathers of waterbird species nesting at Agassiz National Wildlife Refuge, Minnesota

Comparison	*R*-statistic	
	Eggs 2001[a]	Feathers 1999[b]
Species effect	0.556	0.940
Eared grebe/Franklin's gull	0.212	0.920
Eared grebe/night-heron	0.508	0.872
Eared grebe/pied-billed grebe	0.808	—[c]
Franklin's gull/night-heron	0.450	1.00
Franklin's gull/pied-billed grebe	0.772	—
Night-heron/pied-billed grebe	0.668	—

[a] Analysis included Al, Cu, Fe, Hg, Mg, Mn, Se, Sr, and Zn.
[b] Analysis included Al, Cd, Cu, Fe, Hg, Mg, Mn, Se, Sr, and Zn.
[c] Pied-billed grebe feathers were not analyzed in 1999.

Based on mean comparisons, among-species differences in Se and metal concentrations in livers identified in the ANOSIM analysis were influenced by higher concentrations of Cu and Hg in black-crowned night-herons, higher concentrations of Mn in eared grebes, and higher concentrations of Se and lower concentrations of Zn in Franklin's gulls (Tables 5 and 6). Means for each species by year are presented for Hg and Se (Table 6) because of a significant species by year interaction; other metals (Table 5) did not have a significant interaction and means are presented for each species. Yearly differences were influenced by higher concentrations of Cu and lower concentrations of Mn in 2001 than in 1998 and lower concentrations of Fe in 1999 than in either 2001 or 1998 (data not shown).

Discussion

Cadmium

Cadmium concentrations in waterbird eggs, livers, and feathers collected at Agassiz during this study were low. Cd was not detected (detection limit = 0.1 μg/g dry weight) in any eggs. The maximum Cd liver concentration was 0.2 μg/g dry weight, less than one-tenth of the concentration considered elevated (>3 μg/g dry weight; Scheuhammer 1987). Cd concentrations in feathers were similar to the values reported for gull and tern feathers (median = 0.1 μg/g dry weight, range = 0.03 – 0.4 μg/g dry weight; Burger 1996). Additionally, Cd concentrations in Franklin's gull chick feathers collected at Agassiz in 1999 (geometric mean = 0.1 μg/g dry weight, this study) were low and comparable to Franklin's gull chick feathers collected at Agassiz in 1994 (geometric mean = 0.13 μg/g dry weight; Burger and Gochfeld 1996a).

The extreme Cd concentrations in eared grebe eggs from Agassiz reported for 1994 (mean = 3.5 μg/g dry weight; Burger and Gochfeld 1996a) were not observed in later years, either in this study (<0.1 μg/g dry weight) or in another study of eared grebe eggs at Agassiz during 1997, 1998, and 1999 (Burger and Eichhorst 2005; average = <0.1 μg/g dry weight for each year). The elevated Cd concentrations in

Table 2. Se and metal concentrations in recently laid eggs of four waterbird species collected at Agassiz National Wildlife Refuge, Minnesota in 2001 ($n = 5$ samples each)

	Geometric mean (µg/g dry weight) and 95% confidence limits				
Element	Franklin's gull	Black-crowned night-heron	Eared grebe	Pied-billed grebe	Analysis of variance p
Aluminum	3.3 A[a] 1.7–6.2	4.5 A 2.8–7.3	2.4 A 1.9–2.9	2.2 A 1.9–2.7	0.12
Barium	2.7 2.1–3.7	—[b]	—	—	—
Copper	2.6 B 2.4–2.8	4.4 A 3.8–5.0	2.4 B 2.2–2.7	2.6 B 2.4–2.8	<0.0001
Iron	93 A 83–104	93 A 77–112	100 A 88–115	121 A 101–146	0.10
Mercury	0.24 B 0.19–0.29	0.29 B 0.22–0.39	0.11 C 0.09–0.14	0.75 A 0.51–1.10	<0.0001
Magnesium	432 A 404–461	422 A 361–493	383 A 336–436	392 A 365–420	0.41
Manganese	2.0 B 1.7–2.3	3.5 A 2.5–5.0	2.7 AB 2.0–3.6	3.8 A 3.7–3.8	0.006
Selenium	2.5 AB 2.0–3.2	2.7 A 2.1–3.5	3.4 A 2.9–4.0	1.7 B 1.5–2.0	0.002
Strontium	2.1 A 1.6–2.7	1.2 B 0.9–1.7	1.5 AB 1.3–1.7	1.4 AB 1.3–1.6	0.02
Zinc	52 A 48–55	45 A 39–53	45 A 41–50	50 A 46–55	0.25

[a] Means sharing the same letter among species are not significantly different.
[b] Dashes indicate that no mean was calculated because less than 50% of samples had detectable concentrations.

Table 3. Se and metal concentrations in chick feathers of three waterbird species collected at Agassiz National Wildlife Refuge, Minnesota in 1999 ($n = 5$ samples each)

	Geometric mean (µg/g dry weight) and 95% confidence limits			
Element	Franklin's gull	Black-crowned night-heron	Eared grebe	Analysis of variance p
Aluminum	64.1 A[a] 49.2–83.5	11.3 B 8.3–15.3	18.6 B 13.2–26.3	<0.0001
Barium	1.5 A 1.3–1.7	—[b]	0.9 A 0.6–1.6	0.13
Cadmium	0.1 A 0.1–0.2	0.1 A 0.1–0.1	0.1 A 0.1–0.3	0.41
Copper	6.1 AB 5.4–6.8	5.0 B 4.3–5.8	8.0 A 6.6–10.0	0.005
Iron	81 A 60–108	37 B 35–38	29 B 25–33	<0.0001
Mercury	0.4 B 0.3–0.6	1.6 A 1.4–1.8	1.2 A 1.0–1.4	<0.0001
Magnesium	467 B 410–533	634 A 602–668	390 B 347–440	0.0001
Manganese	5.2 AB 4.2–6.5	2.6 B 2.0–3.4	7.4 A 4.4–12.5	0.006
Selenium	3.0 A 2.7–3.3	2.2 A 1.9–2.5	0.9 B 0.6–1.5	0.0003
Strontium	0.9 A 0.7–1.1	0.7 A 0.5–1.0	1.0 A 0.8–1.2	0.34
Zinc	247 A 233–262	198 B 178–221	236 A 230–241	0.003

[a] Means sharing the same letter among species are not significantly different.
[b] Dashes indicate that no mean was calculated because less than 50% of samples had detectable concentrations.

black-crowned night-heron chick feathers collected at Agassiz in 1994 (geometric mean = 1.3 µg/g dry weight; Burger and Gochfeld 1996a) were not observed in feathers collected in this study in 1999 (geometric mean = 0.1 µg/g dry weight).

Chromium

Chromium concentrations in waterbird eggs, livers, and feathers collected at Agassiz during this study were low. The

Table 4. Analysis of similarity for Se and metal patterns in livers of three waterbird species nesting at Agassiz National Wildlife Refuge, Minnesota in 1998, 1999, and 2001

Comparison[a]	*R*-statistic
Two-way crossed analysis	
Species given year effects	0.525
Eared grebe/Franklin's gull	0.484
Eared grebe/night-heron	0.504
Franklin's gull/night-heron	0.683
Year given species effects	0.381
1998/1999	0.308
1998/2001	0.309
1999/2001	0.527

[a] Analysis included Cu, Fe, Hg, Mg, Mn, Se, and Zn.

median Cr concentration in fish-eating bird eggs reported in 21 studies was 0.2 μg/g wet weight (Burger 2002). All of the waterbird eggs colleted in this study were below that concentration. The maximum Cr concentration in chick livers was 0.2 μg/g dry weight, well below a level associated with Cr contamination (>4.0 μg/g dry weight; Eisler 2000). Finally, Cr was not detected in waterbird feathers (detection limit = 0.5 μg/g dry weight) and the detection limit was considerably lower than the value reported for birds in general (median = 8.1 μg/g dry weight, range = 0–18 μg/g dry weight; Burger 1996).

Chromium concentrations in Franklin's gull eggs and chick livers collected during this study were not elevated at Agassiz as reported in 1994 (egg mean = 3.1 μg/g dry weight, Burger and Gochfeld 1996b; liver mean = 16.2 μg/g dry weight, Burger and Gochfeld 1999). The maximum Cr concentration in five Franklin's gull eggs in this study was 0.3 μg/g dry weight. Only 5 of 15 Franklin's gull chick livers had detectable concentrations of Cr (detection limit was <0.5 μg/g dry weight in 1998 and 1999 and 0.1 μg/g dry weight in 2001); the maximum concentration was < 0.2 μg/g dry weight.

Mercury

Mercury concentrations in waterbird eggs, livers, and feathers at Agassiz were not at toxic levels. Mercury concentrations in bird eggs associated with impaired reproduction vary from 2 to 8 μg/g dry weight (assuming 75% moisture; Thompson 1996). In contrast, mean Hg concentrations in fresh eggs were <0.8 μg/g dry weight; the maximum concentration was 1.4 μg/g dry weight. Hg concentrations in waterbird chick livers (<1.0 μg/g dry weight) were well below a level considered toxic (20–30 μg/g wet weight; Thompson 1996). Mean feather concentrations for Franklin's gull (geometric mean = 0.4 μg Hg/g dry weight), black-crowned night-heron (1.6 μg/g dry weight), and eared grebe (1.2 μg/g dry weight) were less than the overall median for 180 studies of Hg concentrations in feathers of birds (2.1 μg/g dry weight; Burger 1996).

Mercury concentrations in eared grebe eggs were comparable to a separate sample of eggs collected at Agassiz in 1999 by Burger and Eichhorst (2005). The geometric mean concentration of Hg in eared grebe eggs collected in 2001 at Agassiz in this study was 0.11 μg/g dry weight. The geometric mean concentration of 13 eared grebe eggs collected from Agassiz in 1999 and analyzed at the Elemental Analysis Laboratory of the Environmental and Occupational Health Sciences Institute (Piscataway, NJ) was 0.19 μg/g dry weight (Burger and Eichhorst 2005).

Selenium

Selenium concentrations in waterbird eggs, livers, and feathers at Agassiz were not at toxic levels. A threshold for reproductive problems in birds is believed to be associated with Se concentrations in excess of 12 μg/g dry weight (3 μg/g wet weight, assuming 75% moisture) in eggs (Heinz 1996). Geometric mean Se concentrations in waterbird eggs at Agassiz were below 3.5 μg/g dry weight. Liver concentrations of egg-laying females >12 μg Se/g dry weight might impair reproduction, and liver concentrations >40 μg Se/g dry weight might have important sublethal effects (assuming 75% moisture; Heinz 1996). Geometric mean Se concentrations in waterbird chick livers at Agassiz were below 5 μg/g dry weight. Mean Se feather concentrations for Franklin's gull (geometric mean = 3.0 μg/g dry weight), black-crowned night-heron (2.2 μg/g dry weight), and eared grebe (0.9 μg/g dry weight) at Agassiz in 1999 were similar to the overall median for 42 studies reporting Se concentrations in bird feathers (2.2 μg/g dry weight; Burger 1996).

Other Metals

None of the other metals present in waterbird eggs, livers, or feathers were at toxic levels. Concentrations of metals in waterbird eggs and livers were comparable or lower than collections from other US locations. As, Cu, Fe, Pb, Mn, and Ni concentrations in feathers from waterbirds collected at Agassiz in 1999 were similar or lower than the median value reported from multiple bird studies conducted worldwide (Burger 1996). Mean Zn concentrations in feathers of all three species were higher than previously reported for birds (Burger 1994). Zinc concentrations in feathers (geometric means 198–247 μg/g dry weight), however, were not considered elevated (>400 μg/g dry weight; Puls 1994).

Conclusion

The unusually high Cd concentrations in eared grebe eggs (3.5 μg/g dry weight, Burger and Gochfeld 1996a), Cd concentrations in black-crowned night-heron chick feathers (1.3 μg/g dry weight, Burger and Gochfeld 1996a), Cr concentrations in Franklin's gull eggs (3.1 μg/g dry weight; Burger and Gochfeld 1996b), and Cr concentrations in Franklin's gull chick livers (16.2 μg/g dry weight, Burger and Gochfeld 1999) reported at Agassiz in 1994 were not confirmed in the present study. In the present study, Cd was not detected (<0.1 μg/g dry weight) in eared grebe eggs, Cd averaged

Table 5. Se and metal concentrations in livers of chicks of three waterbird species collected at Agassiz National Wildlife Refuge, Minnesota in 1998, 1999, and 2001 (n = 5 samples for each species and year).

	Geometric mean (μg/g dry weight) ± 95% confidence limits			
Element	Franklin's gull	Black-crowned night-heron	Eared grebe	Analysis of variance p
Copper	15.4 B[a]	20.5 A	14.9 B	0.007
	13.7–17.3	16.3–25.7	13.9–16.0	
Iron	410 A	329 A	322 A	0.14
	340–495	273–397	236–442	
Magnesium	733 A	704 A	709 A	0.41
	707–760	665–744	681–738	
Manganese	15.6 B	11.6 C	18.7 A	<0.0001
	14.6–16.7	10.5–12.8	16.6–21.1	
Zinc	80 B	112 A	100 A	0.0009
	77–83	95–132	91–109	

Note: Results of a two-way analysis of variance (species year species * year) where there was no significant interaction (species * year). Year differences are not shown.

[a] Means sharing the same letter among species are not significantly different.

Table 6. Hg and Se concentrations in livers of chicks of three waterbird species collected at Agassiz National Wildlife Refuge, Minnesota in 1998, 1999, and 2001 (n = 5 samples for each species and year).

	Geometric mean (μg/g dry weight) ± 95% confidence limits			
Element	Year	Franklin's gull	Black-crowned night-heron	Eared grebe
Mercury	1998	0.13 E[a]	0.70 AB	0.28 CD
		0.10–0.16	0.48–1.00	0.20–0.39
	1999	0.14 DE	0.80 A	0.37 BC
		0.12–0.16	0.67–0.95	0.32–0.43
	2001	0.23 CD	0.80 A	0.24 CD
		0.19–0.27	0.66–0.97	0.15–0.40
Selenium	1998	4.5 AB	3.4 ABC	2.4 C
		4.0–5.0	2.7–4.2	2.2-2.6
	1999	4.9 A	3.2 BC	3.4 ABC
		4.4–5.3	2.7–3.9	3.1–3.7
	2001	4.3 AB	3.7 AB	3.5 ABC
		3.8–4.8	3.0–4.6	2.9–4.4

Note: Results of a one-way analysis of variance following a two-way analysis of variance (species year species * year) where there was a significant interaction (species * year).

[a] Means sharing the same letter among all species and years are not significantly different.

0.1 μg/g dry weight in black-crowned night-heron chick feathers, the maximum Cr concentration was 0.3 μg/g dry weight in Franklin's gull eggs, and Cr concentrations in Franklin's gull chick livers were <0.2 μg/g dry weight.

The high Cd and Cr concentrations at Agassiz in 1994 are difficult to explain. Burger and Gochfeld (1996a) and Burger and Eichhorst (2005) speculated that the high levels of Cd reported in eared grebe eggs (Burger and Gochfeld 1996a) might have occurred because egg laying in 1994 began soon after arrival at Agassiz and, therefore, levels might have reflected exposure on wintering or migratory areas. Unfortunately, no data on arrival and laying time were provided to test this hypothesis. Because contaminant concentrations in chicks mainly represent local exposure, this argument cannot be used to explain the high Cd concentrations in black-crowned night-heron chick feathers (Burger and Gochfeld 1996a) or the high Cr concentrations in Franklin's gull chick livers (Burger and Gochfeld 1999). The elevated levels of Cr in the Franklin's gull eggs might be explained as a reporting error. Mean Cr concentrations in the same cohort of Franklin's gull eggs were reported as 3.1 μg/g dry weight in Burger and Gochfeld (1996b) but only 0.6 μg/g dry weight in Burger and Gochfeld (1996a).

The low Cd and Cr concentrations reported here are consistent with recent studies of grebes (Burger and Eichhorst 2005) and tree swallows (Custer *et al*. 2006) collected at Agassiz. Cd concentrations in eared grebe eggs collected during 1997, 1998, and 1999 averaged 0.033, 0.026, and 0.001 μg/g dry weight, respectively (Burger and Eichhorst 2005). Cd concentrations in tree swallow eggs collected during 1998–2001 at Agassiz were generally (110 of 115 eggs) less than the detection limit of 0.1 μg/g dry weight (Custer *et al.* 2006). Cr concentrations in tree swallow nestling livers collected during 1998–2001 at Agassiz were generally (90 of 105 livers) less than the detection limit (0.1 [2001] to 0.5 [1998–2000] μg/g dry weight) (Custer *et al.* 2006). These studies in conjunction with the present study indicate that Se and metal concentrations, including Cd and Cr, in bird eggs and chicks collected at Agassiz were not at toxic levels.

Acknowledgments. We thank Craig Beckman, Paul Dummer, Gary Huschle, and Jean Mengelkoch for field assistance, Larry Schmidt for advice on feather cleaning methods, and Margaret Anderson for allowing us to work on Agassiz National Wildlife Refuge. This study was funded through On-Refuge Contaminants Investigations of the US Fish and Wildlife Service.

References

Burger J (1994) Metals in avian feathers: bioindicators of environmental pollution. Rev Environ Toxicol 5:203–311

Burger J (1996) Heavy metal and selenium levels in feathers of Franklin's gulls in interior North America. Auk 113:399–407

Burger J (2002) Food chain differences affect heavy metals in bird eggs in Barnegat Bay, New Jersey. Environ Res 90:33–39

Burger J, Eichhorst B (2005) Heavy metals and selenium in grebe eggs from Agassiz National Wildlife Refuge in northern Minnesota. Environ Monit Assess 107:285–295

Burger J, Gochfeld M (1996a) Heavy metal and selenium levels in birds at Agassiz National Wildlife Refuge, Minnesota: food chain differences. Environ Monit Assess 43:267–282

Burger J, Gochfeld M (1996b) Heavy metal and selenium levels in Franklin's gull (*Larus pipixican*) parents and their eggs. Arch Environ Contam Toxicol 30:487–491

Burger J, Gochfeld M (1999) Heavy metals in Franklin's gull tissues: age and tissue differences. Environ Toxicol Chem 18:673–678

Clarke KR, Warwick RM (1994) Change in marine communities: An approach to statistical analysis and interpretation, 2nd ed. Plymouth Marine Laboratories, Plymouth, UK

Custer CM, Custer TW, Warburton D, Hoffman D, Bickham J, Matson C (2006) Trace element concentrations and bioindicator response in tree swallows from northwestern Minnesota. Environ Monit Assess 118:247–266

Eisler R (2000) Handbook of chemical risk assessment: Health hazards to humans, plants, and animals. Volume 1, Chromium, Lewis Publishers, Boca Raton, FL, pp 45–92

EPA (Environmental Protection Agency) (1991) Methods for determination of metals in environmental samples, Report No. EPA-600/4-91-010, Environmental Monitoring Systems Laboratory, Office of Research and Development

Furness RW (1996) Cadmium in birds. In: Beyer WN, Heinz GH, Redmond-Norwood AW, (eds) Environmental contaminants in wildlife, interpreting tissue concentrations. Lewis Publishers, New York, pp 389–404

Hays H, LeCroy M (1971) Field criteria for determining incubation stage in eggs of the common tern. Wilson Bull 83:425–429

Heinz GH (1996) Selenium in birds. In: Beyer WN, Heinz GH, Redmon-Norwood AW, (eds) Environmental contaminants in wildlife, interpreting tissue concentrations. Lewis Publishers, New York, pp 447–458

Puls R (1994) Mineral levels in animal health, diagnostic data, 2nd ed. Sherpa International, Clearbrook, BC, Canada

Scheuhammer AM (1987) The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: a review. Environ Pollut 46:263–295

Thompson DR (1996) Mercury in birds and terrestrial mammals. In: Beyer WN, Heinz GH, Redmon-Norwood AW, (eds) Environmental contaminants in wildlife, interpreting tissue concentrations. Lewis Publishers, New York, pp 341–356