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HEAVY METALS AND SELENIUM IN GREBE EGGS FROM AGASSIZ NATIONAL WILDLIFE REFUGE IN NORTHERN MINNESOTA

JOANNA BURGER[1,*] and BRUCE EICHHORST[2,3]
[1]*Division of Life Sciences, Rutgers University, Piscataway, New Jersey, U.S.A.;* [2]*Department of Biology, University of Nebraska-Kearney, Kearney, Nebraska, U.S.A.;* [3]*Department of Biology, University of North Dakota, Grand Forks, North Dakota, U.S.A.*
*(*author for correspondence, e-mail: burger@biology.rutgers.edu)*

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Abstract. Metal levels in eggs can often be used as an indicator of exposure and of potential effects. In previous work at Agassiz National Wildlife Refuge, northwestern Minnesota, the levels of several heavy metals were shown to be significantly higher in the eggs of eared grebes (*Podiceps nigricollis*) compared to those in the eggs of Franklin's gulls (*Larus pipixcan*), black-crowned night-herons (*Nycticorax nycticorax*) and double-crested cormorants (*Phalacrocorax auritus*, except for mercury). In the present study we test the hypothesis that there are no differences in the levels of heavy metals in eggs of three species of grebes nesting at Agassiz National Wildlife Refuge (1997, 1999). There were significant differences in levels of selenium, manganese and mercury in the eggs of the grebes collected in 1997, with pied-billed grebe (*Podilymbus podiceps*) having significantly higher levels of manganese and mercury, and significantly lower levels of selenium, than eared or red-necked grebes (*Podiceps grisegena*). In 1999, pied-billed grebes had significantly higher levels of mercury, but lower levels of selenium and tin than the other species. The only pattern that was significant and consistent among years was selenium; in both years pied-billed grebes had lower levels than the other species. For eared grebes, there was a decline from 1997 to 1998, and again to 1999 for arsenic, cadmium, and selenium. Levels of mercury in the eggs of grebes were not as high, however, as those found in cormorants or night-herons sampled in 1994 at Agassiz National Wildlife Refuge. There were few consistent patterns in the relationships among metals in eared grebe eggs (with the largest sample sizes). The possible reasons for the high levels of some metals in eggs of grebes are unknown, but presumably egg levels represent exposure on the wintering grounds or migratory routes. In comparison to eggs of other birds: 1) the mean levels for manganese were at the high end of the range, and the mean was an order of magnitude higher than the median for the studies examined, 2) mean levels were above the median in the eggs of other birds for lead (red-necked grebe), mercury (pied-billed grebe) and selenium (eared and red-necked grebe).

Keywords: cadmium, eared grebe, grebes, heavy metals, mercury, pied-billed grebe, red-necked grebe

1. Introduction

Contaminants can bioaccumulate over time to reach toxic or even lethal levels unless organisms can excrete them or detoxify them. Many contaminants are persistent in nature, and can accumulate in tissues such that levels increase with the age of a bird and with each succeeding step in the food chain. Concentrations in long-lived and top-level carnivores can reach levels that are much higher than lower trophic levels (van Straalen and Ernst, 1991; Burger *et al.*, 1992; Sundlof *et al.*, 1994; Burger, 2002). Seabirds and many other colonial species are particularly vulnerable because they are long-lived, are often high on the trophic chain, and live in coastal or marine environments where contaminant levels may be high (Burger, 1993).

In earlier work at Agassiz National Wildlife Refuge, Burger and Gochfeld (1996) showed that eared grebes (*Podiceps nigricollis*) had higher levels of all metals (except mercury) in their eggs than American coots (*Fulica americana*), Franklin's gulls (*Larus pipixcan*), black-crowned night-herons (*Nycticorax nycticorax*) and double-crested cormorants (*Phalacrocorax auritus*). This was not expected on the basis of food chain relationships. Only mercury showed a pattern of increasing levels with trophic level on the food chain.

In this paper we examine the levels of arsenic, cadmium, chromium, lead, manganese, mercury, and selenium in the eggs of eared, pied-billed (*Podilymbus podiceps*), red-necked (*Podiceps grisegena*), and western grebes (*Aechmophorus occidentalis*) from Agassiz National Wildlife Refuge in northwestern Minnesota in 1997. Because of the high levels in eggs in 1997, we examined metals in eggs again in 1998 (for eared grebe), and in 1999 for eared, pied-billed and red-necked grebes. One objective was to determine whether the grebes showed a consistent pattern among years, whether the patterns were similar among different grebe species, and whether there were consistent temporal patterns in the eggs of eared grebes in the three years.

2. Methods

Under appropriate federal and state permits eggs were collected at Agassiz National Wildlife Refuge, Marshall County, Minnesota (48°21′N, 95°57′W) from the nests of eared, pied-billed, red-necked and western grebes in 1997, for the former three species in 1999, and from eared grebes in 1998. One egg each was collected from all nests located during the early part of the nesting season. Eggs were refrigerated until they were shipped to the Environmental and Occupational Health Sciences Institute for analysis.

All specimens were analyzed in the Elemental Analysis Laboratory of the Environmental and Occupational Health Sciences Institute in Piscataway. Eggs were digested individually in warm nitric acid mixed with the addition of 30% hydrogen peroxide, and subsequently diluted with deionized water. Samples were subsequently diluted in deionized water. Mercury was analyzed by cold vapor technique, and the other elements were analyzed by graphite furnace atomic absorption (Burger and Gochfeld, 1991). All concentrations are expressed in ng/g (ppb) on a dry weight basis using weights obtained from air-dried specimens.

Detection limit ranges were: 0.02 ppb for cadmium, 0.08 ppb for chromium, 0.15 ppb for lead, 0.09 ppb for manganese, 0.2 ppb for mercury, and 0.7 ppb for selenium. All specimens were analyzed in batches with known standards, calibration standards, and spiked specimens. Recoveries ranged from 88% to 102%. Batches with recoveries of less than 85% were reanalyzed. The coefficient of variation on replicate, spiked samples ranged up to 10%.

Non-parametric Wilcoxon chi square tests were used to examine differences among species, and Duncan Multiple range tests were performed to distinguish significant differences between species. Western grebes are not included in the statistical analyses because of their small sample sizes. Both arithmetic and geometric means are given for eggs to facilitate comparisons with other studies.

3. Results

There were no significant species differences in concentrations of arsenic, chromium, and lead in eggs from 1997 (Table I). Pied-billed grebes had significantly higher levels of manganese and mercury, and significantly lower levels of selenium, in their eggs than did the other species. Eared grebes had significantly higher levels of cadmium in their eggs than the other species.

In 1999, there were no significant species differences in concentrations of cadmium, manganese and mercury in eggs (Table II). However, eared grebes had significantly higher levels of arsenic, and to a lesser extent chromium than the other species, and red-necked grebes had higher levels of lead than the other species (Table II). The other pattern that was significant and consistent among years was selenium: in both years pied-billed grebes had lower levels than the other species (Tables I and II).

Because of the high levels of metals in eggs in 1997, we had also collected eggs of eared grebes in 1998, allowing for the analysis of the temporal patterns in metals (Table III). Although there were significant differences among years for all metals, only arsenic, cadmium, chromium, and selenium showed a significant decline from 1997–1999.

For eared grebes, the species with the largest sample sizes, there were some significant correlations among metals for eggs (Table IV). However, there were few patterns: lead and mercury were negatively correlated with manganese, arsenic and selenium were positively correlated, and selenium and chromium were positively correlated with manganese (Table IV). For the other species, there was only one significant correlation among metals for pied-billed grebe eggs (arsenic and selenium: $r^2 = 0.46$, $P < 0.005$), and only one significant correlation among metals for red-necked grebe eggs (lead and mercury: $r^2 = 0.53$, $P < 0.0009$).

TABLE I
Heavy metals in grebe eggs (ppb, dry weight) from Agassiz National Wildlife Refuge in 1997

	Eared grebe	Pied-billed grebe	Red-necked grebe	Western grebe	Wilcoxon X^2 (p)[a]
Sample Size	26	21	21	2	
Arsenic	174 ± 28 (A) (80.3)	156 ± 26 (A) (105)	224 ± 26 (A) (187)	148 ± 8 (148)	NS
Cadmium	32.5 ± 9 (A) (12.9)	12 ± 2 (B) (8.0)	22 ± 5 (A) (12.1)	9 ± 3 (8.63)	4.89 (0.09)
Chromium	1700 ± 555 (A) (1050)	863 ± 133 (A) (641)	2910 ± 1360 (A) (1110)	1650 ± 402 (1600)	NS
Lead	211 ± 36 (A) (134)	155 ± 18 (A) (103)	232 ± 50.7 (A) (146)	41.2 ± 40.9 (5)	NS
Manganese	3440 ± 154 (B) (3340)	6620 ± 459 (A) (6340)	3100 ± 276 (B) (2910)	3730 ± 1180 (3540)	37.8 (0.0001)
Mercury	215 ± 26 (B) (184)	674 ± 108 (A) (557)	283 ± 23 (B) (269)	355 ± 215 (282)	33.6 (0.0001)
Selenium	3060 ± 85 (A) (3040)	1620 ± 76 (B) (1590)	2960 ± 119 (A) (2920)	2420 ± 74.5 (2410)	42.5 (0.0001)

Note. Given are means ± one standard error. Like letters are not significantly different when groups are considered (Duncan Multiple Range Test). Western grebes were not included in statistical analysis because of small sample sizes. Geometric means in parentheses. NS = not significant.
[a]Western grebe not included in analysis.

TABLE II
Heavy metals in grebe eggs (ppb, dry weight) from Agassiz National Wildlife Refuge

	Eared grebe	Pied-billed grebe	Red-necked grebe	Wilcoxon X^2 (p)
Sample size	13	7	16	
Arsenic	46 ± 12 (A) (12)	9 ± 5 (B) (1)	26 ± 9 (A, B) (1.6)	4.6 (0.06)
Cadmium	1 ± 0.2 (B) (0.7)	2 ± 0.90 (A) (0.8)	1 ± 0.24 (A,B) (0.9)	NS
Chromium	216 ± 62 (A) (150)	119 ± 14 (A, B) (114)	83 ± 10 (B) (74)	7.2 (0.03)
Lead	81 ± 19 (A, B) (134)	27 ± 8 (B) (11)	379 ± 136 (A) (146)	8.8 (0.01)
Manganese	3443 ± 408 (A) (3113)	3102 ± 290 (A) (3001)	4371 ± 707 A) (3931)	NS
Mercury	222 ± 40 (B) (190)	409 ± 119 (A) (341)	247 ± 30 (B) (221)	NS
Selenium	1861 ± 98 (A) (1830)	1154 ± 97 (B) (1130)	1926 ± 195 (A) (1811)	11 (0.003)
Tin	633 ± 238 (A) (171)	224 ± 91 (B) (32)	312 ± 60 (A) (173)	NS

Note. Given are means ± standard error for eggs collected in 1999. Like letters are not significantly different when groups are considered (Duncan Multiple Range Test). Geometric means in parentheses. NS = not significant.

TABLE III
Heavy metals in eared grebe eggs for three consecutive years 1997, 1998 and 1999

(N)	1994[a]	1997 26	1998 24	1999 13	Wilcoxon (p)
Metals					
Arsenic		174 ± 28.0 80 (A)	74 ± 9 51 (B)	46 ± 12.0 12 (B)	11 (0.005)
Cadmium	3470 ± 713	33.00 ± 9.00 13 (A)	26.00 ± 7.00 14 (A)	1 ± 0.2 0.7 (B)	26 (0.0001)
Chromium	676 ± 51	1701 ± 555 1053 (A)	143 ± 25 80 (B)	216 ± 62 150 (B)	40 (0.0001)
Lead	245 ± 41	211 ± 36 134 (A, B)	350 ± 71 271 (A)	81 ± 19 40 (B)	19 (0.0001)
Manganese	4160 ± 304	3436 ± 154 3339 (A)	2021 ± 121 1926 (B)	3443 ± 408 3113 (A)	24 (0.0001)
Mercury	429 ± 51	215 ± 26 184 (A)	298 ± 31 250 (A)	222 ± 40 190 (A)	6 (0.06)
Selenium	3120 ± 304	3064 ± 85 3035 (A)	2080 ± 107 1969 (B)	186110 ± 98 1830 (B)	41 (0.0001)
Tin			793 ± 80 705 (A)	502 ± 136 136 (A)	6 (0.01)

Note. Levels from 1994 provided arithmetic means for comparison. Given are means and S.E. Geometric means with Duncan categories in parentheses. Years sharing a letter do not differ significantly. Results are in ppb (dry weight).
[a]Burger and Gochfeld (1996).

TABLE IV
Correlations of metals within eggs of eared grebe '1997–1998' (above diagonal) and feathers of eared grebe '1997' (below diagonal)

	Lead	Cadmium	Selenium	Chromium	Manganese	Arsenic	Mercury
Lead	–	NS –	NS	NS	−0.268 (0.008)	NS	NS
Cadmium			0.27 (0.002)	NS	NS	NS	NS
Selenium			–	0.37 (0.0001)	0.17 (0.04)	0.28 (0.001)	NS
Chromium				–	0.34 (0.0001)	NS	NS
Manganese					–	NS –	−0.26 (0.003)
Arsenic							NS
Mercury							–

Note. Given are the non-parametric Kendall-tau (p). NS = not significant.

4. Discussion

4.1. Birds as bioindicators

Birds are often used to monitor heavy metals because some species are at the top of the food chain, and might be expected to accumulate high levels. They excrete contaminants directly or they can sequester them in their feathers, and females can excrete them in their eggs and eggshells (Fimreite *et al.*, 1982; Burger and Gochfeld, 1991, 1993; Burger, 1993). In addition, Kim *et al.* (1996) recently suggested that some pelagic seabirds (albatrosses and petrels) are capable of demethylating methymercury in the liver, and storing mercury in an immobilizable inorganic form.

For most birds, feathers and eggs can serve as an indicator of internal contamination (Goede and deBruin, 1984, 1986; Furness *et al.*, 1986; Burger, 1993; Becker *et al.*, 1998; Burger and Gochfeld, 2003). These levels can then be used to assess whether there are potential reproductive deficits in these populations. The relatively high levels of metals found in eggs of eared grebes in a previous study at Agassiz (Burger and Gochfeld, 1996) suggested that grebes should be examined in more detail.

This research was designed to examine the possible use of grebes as bioindicators, to determine whether the high levels of heavy metals in eared grebes previously observed were an anomaly, or typical of the species at Agassiz. Levels in the eggs usually reflect local exposure.

In 1994, eared grebes had the highest levels in their eggs of all metals except mercury (Burger and Gochfeld, 1996). In 1994, eared grebes may have laid soon after arrival (while they did not in the other years), suggesting that levels in eggs might have reflected exposure on the wintering grounds at the Salton Sea. The levels in the eggs of eared grebes collected from 1997 were the same order of magnitude to those in eggs from 1994 (except cadmium, which was two orders of magnitude lower in 1997). The levels in eared grebe eggs declined significantly from 1997 to 1999 for arsenic, cadmium, chromium and selenium; mercury was lower in all three years, but the declines in the other species were negligible.

However, pied-billed grebe eggs (which were not collected in 1994) had significantly higher levels of mercury (1997, 1999) and manganese (1997 only) than eared grebe eggs. This suggests that further work with pied-billed grebes should be conducted at Agassiz and elsewhere, to determine whether this is typical generally, or whether exposure is higher for the birds at Agassiz.

4.2. Metal levels in eggs

This research indicated that pied-billed grebes had significantly higher levels of manganese (1997 only) and mercury (both years), and lower levels of selenium, in their eggs than the other species; eared grebes had significantly higher levels of cadmium than the other species only in 1997. Thus, even within the grebes at Agassiz there are interspecific differences in egg levels. These differences could result from different lengths of time at Agassiz (and thus different levels of exposure), or consumption of different food with different levels of metals. In general, red-necked grebes eat a variety of small fish, aquatic and land insects, tadpoles, crustaceans, mollusks and aquatic worms; eared grebes eat primarily aquatic and land insects and their larvae; pied-billed grebes eat primarily fish, crayfish and other crustaceans, and insects (Palmer, 1962). The relatively higher levels of contaminants in the eggs of pied-billed grebes may indicate that they eat greater amounts of fish than the other species (or eat fish for a longer period of time). Fish are generally higher on the food chain, and usually accumulate significantly more heavy metals than invertebrates.

4.3. Comparisons of eggs with other species

Although there are fewer studies examining the levels of heavy metals in eggs compared to levels in feathers, a significant literature is now available. Although there were some patterns in metal concentrations, the egg data for grebes from Agassiz exhibited variation among species and years. However, some comparisons can be made for the three grebe species examined in both years, levels of all metals in 1999 were either lower than or similar to those of 1997. In the comparisons below, I compare levels in 1999 with those from a range of other studies (after Burger, 2002). Arsenic and tin are not examined because there are too few studies in the literature.

The mean of cadmium concentrations in eggs for 32 studies indicated a range of 2–600 ppb, with a median of 14, indicating that cadmium levels in eggs of all three grebe species are within the usual range for bird eggs. Eared grebes may be ingesting sediment that had high levels of cadmium; cadmium comes from erosion of surface deposits and from anthropogenic sources, such as purification of ores in smelters and mines, as well as from commercial products (Parmeggiani, 1983).

The mean of chromium concentrations in eggs for 21 studies indicated a range of 10–1000 ppb, with a median of 210 ppb, indicating that chromium levels in eggs of all three grebe species are about at the median.

The mean of lead concentrations in eggs for 29 studies indicated a range of 20–6700 ppb, with a median of 190 ppb, indicating that lead levels in eggs of red-necked grebes are above the median, but the other grebe species are lower than the median.

The mean of manganese concentrations in eggs for 13 studies indicated a range of 350–4000, with a median of 510 ppb, indicating that manganese levels in eggs of all three grebe species are at the high end of the mean levels for bird eggs, and are much higher than the median.

The mean of mercury concentrations in eggs for 68 studies indicated a range of 70–7290 ppb, with a median of 340 ppb, indicating that mercury levels in eggs of all three grebe species are close to the median for these studies. Mercury comes from natural erosion of soils and underlying bedrock, as well as from anthropogenic sources (Parmeggiani, 1983). The grebes, however, may have obtained their mercury during the winter, while they were along the coasts. Mercury levels are often higher in marine systems. Further, the periodic flooding of freshwater marshes results in more mobilization of mercury from soil than normally occurs at the bottom of lakes (Zillious *et al.*, 1993; Hudson *et al.*, 1994).

The mean of selenium concentrations in eggs for 13 studies indicated a range of 300–7100 ppb, with a median of 1100 ppb, indicating that selenium levels in eggs of all three grebe species are well within the range, but are close to (pied-billed Grebe) or above the median (the other species).

Overall, the levels of most metals in the eggs of grebes from Agassiz are close to the median for a range of other species. However, there were exceptions: 1) mean levels for lead in red-necked grebes were above the median, 2) mean levels for manganese were at the high end of the range for manganese in other birds, and the mean was an order of magnitude higher than the median for the studies examined (Burger, 2002), 3) mean mercury levels for pied-billed grebe were higher than the median for the studies examined, and 4) although mean selenium levels were well within the range for other species, they were higher than the median in eared and red-necked grebe. Since eggs generally reflect local exposure, the levels of these metals should be monitored to assess continued exposure that could imply adverse effects (especially for manganese).

4.4. Significance

Heavy metals can cause defects in reproductive behavior and decrease reproductive success in birds, as well as decreasing survival at high levels (Burger, 1993). The three metals of concern from this study are mercury, manganese, and cadmium, which were relatively high in the eggs of grebes, both when compared to other species at Agassiz, and when compared to other bird species from elsewhere. Although other metals, such as selenium, have been shown to have adverse effects on survival and reproductive success in birds (Ohlendorf and Harrison, 1986; Ohlendorf *et al.*, 1989), the levels of these metals were within the range reported for other species where no ill effects were noted.

Mercury levels of 1000 ppb in eggs decrease hatchability and produce behavioral effects in hatchlings (Eisler, 1987). Other studies have found adverse effects on hatchability at 500 ppb (Fimreite, 1971). Although the levels in eggs were well below 1000 ppb for most grebe species at Agassiz, the mean for pied-billed grebe was over 600 ppb, cause for some concern. Further, pied-billed grebes had significantly higher levels of mercury in their eggs than eared grebes. The adverse effects of mercury are somewhat counterbalanced by selenium (Molen *et al.*, 1982), thus, the effects of mercury on developing grebe embryos at Agassiz may be lessened.

Cadmium is a non-essential heavy metal. It causes kidney toxicity in vertebrates, as well as causing decreased testis weight and spermatogenesis failure (Richardson *et al.*, 1974; Connors *et al.*, 1975). However, there have not been as great an emphasis on understanding cadmium toxicity in laboratory studies.

Manganese is an essential element that serves as an important co-factor (Drown *et al.*, 1986). Exposure to high concentrations can lead to neurobehavioral and respiratory health effects in laboratory studies of birds and mammals (Ingersoll *et al.*, 1995; Sentuck and Oner, 1996; Burger and Gochfeld, 2001). Unfortunately, the levels of manganese in tissues associated with these adverse effects are not often determined. Thus, the potential effects on the grebes are unknown, but bear examination.

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