METALS AND TRACE ELEMENTS IN EGGS OF AMERICAN WHITE PELICANS AT ANAHO ISLAND, NEVADA 2004

By

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Introduction

A large colony of American white pelicans (*Pelecanus erythrorhynchos*) nests on Anaho Island National Wildlife Refuge, which is on Pyramid Lake, Nevada, at the terminus of the Truckee River. Reproductive success of the colony was monitored in 1996 and a single egg was collected from each of 30 nests for analysis of organochlorine pesticides and metals and trace elements (Wiemeyer et al. 2001). Additional samples collected for residue analysis included: blood and feathers from two different age groups of nestling pelicans; livers from healthy and weak nestlings; liver and muscle samples from adults found dead or debilitated in the area; regurgitate samples from nestling pelicans; and fish from known feeding areas that were frequented by the pelicans. Reproductive success of the Anaho Island colony was normal based on hatching rates of eggs and survival of nestlings. Organochlorine and polychlorinated biphenyl (PCB) residues in eggs were below known effect levels, with biologically insignificant shell thinning. Organochlorine pesticides and PCBs were seldom detected in fish, but organochlorine pesticides were elevated in muscle samples of some adult pelicans. Mercury concentrations in eggs were generally below known effect concentrations, as were concentrations of other metals and trace elements. Metal and trace element concentrations in fish ranged widely for some constituents, with mercury of greatest concern. Microscopic lesions of mercury toxicity were absent in pre-fledging nestlings. Some adult pelicans had elevated mercury concentrations in their livers. However, the potential toxic effects were difficult to evaluate because the proportion of methyl-mercury declined as the total mercury concentrations increased, thereby possibly providing protection from toxicity (Henny et al. 2002).

Pelican nesting success at Anaho Island has fluctuated greatly in the past eight years, from a high of 8,500 juveniles fledged in 1999 to a low of 440 juveniles fledged in 2003. Between 1996 and 1999, an average of 6,700 pelican nests produced 5,650 fledged juveniles, annually. Between 2000 and 2003, the averages dropped to only 4,200 pelican nests producing 630 juveniles each year. Drought conditions are thought to be a primary factor in high rates of juvenile mortality due to starvation. However, there have been lingering concerns regarding the potential adverse effects of mercury on reproduction and survival of American white pelicans nesting at Anaho Island. These concerns are greater in drought years when the birds may use more highly contaminated food sources. These concerns and the recent declines in the nesting population linked with reproductive problems warranted additional study of metals and trace elements, especially mercury, in eggs of pelicans nesting at the colony. The concerns in relation to mercury are heightened by an apparent increase in mercury concentrations in forage fish in Pyramid Lake, including cui-ui (*Chasmistes cujus*), tui chub (*Gila bicolor*), and Tahoe sucker (*Catostomus tahoensis*) between 1996 (Wiemeyer et al. 2001) and 2001 (Slotton and Ayers 2002). The cause of the increase is unknown, but might be related to increased mercury inputs into the lake in relation to the flood of January 1997 on the Truckee River.

The overall purpose of the study was to determine if inorganic contaminants may be responsible for the recent declines in reproductive success of American white pelicans that nest on Anaho Island. The objective of the study was to determine present concentrations of metals and trace elements in eggs from one sub-colony of pelicans at Anaho Island.

Methods

One egg was collected from each of 10 nests during mid-incubation on May 12, 2004. All eggs were collected from the west end of the Bluff sub-colony, with the egg collection occurring at night to preclude predation by California gulls (*Larus californicus*) (Bowman et al. 1994). Sampled nests and an equal number of adjacent un-sampled nests were marked with numbered stakes. The reproductive success of both the sampled and un-sampled nests was determined by observation with a spotting scope from a vantage point above the colony. Eggs were placed in a cushioned container in the field and placed in a refrigerator in the laboratory within 24 hours of collection. Eggs were weighed (grams), and length and breadth were measured (millimeters) with calipers. Eggs were opened with chemically clean stainless steel instruments on May 13 and 14, 2004, and egg contents were placed in chemically-clean glass jars with Teflon lined lids. Egg contents were examined for embryonic development and gross abnormalities and frozen. Egg volumes were not measured due to missing equipment at the time of egg processing. An attempt was made to develop a regression equation predicting egg volume using length, breadth, and volume data from the 1996 pelican eggs; however, the equation did not satisfactorily predict volume from length and breadth data when used against the 1996 data. Total egg weight appeared to more closely predict egg volume and was therefore used in place of the regression equation. Eggshells were gently rinsed with tap water and air dried. Eggshell thickness was measured with a Starrett® 1010M micrometer on June 22, 2004. Specimens for residue analysis were shipped to Laboratory and Environmental Testing, Inc., Columbia, Missouri on June 21, 2004.

Samples were homogenized at the analytical laboratory. Sub-samples were digested using various means, depending on the element to be analyzed. Arsenic and selenium analyses were by hydride generation atomic absorption (AA), mercury analyses were by cold vapor AA, lead analyses were by graphite furnace AA, and the remainder were by inductively coupled plasma (ICP). Quality assurance and quality control (QA/QC) included the analyses of one sample each of blank, duplicate, spike, and reference material. All QA/QC results were satisfactory and were certified by the Patuxent Analytical Control Facility of the U.S. Fish and Wildlife Service, Laurel, Maryland.

All residue concentrations were $\log_{10}$ transformed prior to statistical analysis. One-half of the detection limit was assigned to samples with non-detectable concentrations. Geometric mean concentrations are reported only when $\geq 50\%$ of the samples had detectible concentrations of a given metal or trace element. A SYSTAT® (SYSTAT 2000) program was used in conducting analyses. One-way analysis of variance (ANOVA) was use for comparisons among years and areas. Bonferroni multiple comparison tests were used to determine which samples were significantly different from one another when differences ($p < 0.05$) among samples were found with ANOVA. Data from pelican eggs collected at Malheur National Wildlife Refuge in 1996 were included in statistical analyses because this site was previously used as a reference site (Wiemeyer et al. 2001).

Residue concentrations in eggs were calculated as micrograms per milliliter on the basis of estimated egg volume and converted to a fresh wet weight basis (µg/g) assuming a specific gravity of 1.0 (Stickel et al. 1966).

Results and Discussion

Reproductive Success

The pelican nests that were sampled, as well as adjacent nests, were abandoned within a week of egg collection, precluding analysis of relationships between hatchability and residue concentrations in eggs. Nine of 10 eggs contained viable embryos that were approximately 15 to 22 days old. The remaining egg appeared to be infertile.

Eggshell Thickness

The mean eggshell thickness at Anaho Island in 2004 was unchanged from the overall mean for Area C and Ridge in 1996 and was only 5.9% below the pre-DDT norm (i.e., 0.682 mm; D. Anderson, pers. comm.) (Table 1). This is a biologically insignificant level of shell thinning. Shell thickness in 2004 ranged from 0.588 mm to 0.734 mm.

Concentrations in Eggs

Concentrations of metals and trace elements in eggs were compared among years at Anaho Island, with the two sub-colonies in 1996 considered separately, and with the inclusion of the 1996 data from Malheur National Wildlife Refuge in Oregon (Table 1). Concentrations of aluminum, arsenic, barium, boron, chromium, molybdenum, and nickel were commonly below detection limits, precluding statistical analyses. Geometric mean concentrations of copper, iron, and zinc (excluding copper in Anaho eggs in 1988 due to a higher detection limit) were not significantly different among all years and areas. The geometric mean magnesium concentration was significantly higher in eggs from the Anaho Bluff sub-colony in 2004 than for eggs from the Anaho Ridge sub-colony and the Malheur colony in 1996, but was similar to the mean concentration at the Anaho Area C sub-colony in 1996; Anaho 1988 data were not included in the analysis due to a high detection limit. The geometric mean manganese concentration in the eggs from the Anaho Bluff sub-colony in 2004 was not significantly different from those in eggs collected in 1996 at Anaho and Malheur; the Anaho data from 1988 were not be included in the analysis due to a much higher detection limit. The geometric mean strontium concentration in the 2004 eggs from the Anaho Bluff sub-colony was not significantly different from those from this colony in 1996, but was significantly higher than the mean concentrations found at Anaho in 1988 and at Malheur in 1996.

The geometric mean mercury concentration in eggs from the Anaho Bluff sub-colony in 2004 was not significantly different from those in eggs collected in 1996 at Anaho and Malheur as well as at Anaho in 1988. The mean concentration in 2004 was intermediate between those found at Anaho in 1988 and 1996. The geometric mean mercury concentration in Anaho eggs collected in 1988 was significantly higher than the mean concentrations in the samples from both sub-colonies sampled at Anaho in 1996.

The geometric mean selenium concentration in Anaho eggs collected from the Bluff sub-colony in 2004 was significantly lower than those found in eggs from both Anaho sub-colonies sampled in 1996 and was not significantly different from that found in the Anaho eggs collected in 1988 nor that in the Malheur eggs collected in 1996.

Concentrations in Eggs in Relation to Adverse Effects

Concentrations of metals and trace elements in American white pelican eggs were compared to background levels (BL), no observed effect levels (NOEL), lowest observed effect levels (LOEL), and dose-response thresholds (DRT) that were published by Seiler et al. (2003). Concentrations in Seiler et al. (2003) were reported on a dry weight basis and were divided by five, based on an average moisture content in eggs of 80%, to obtain estimated wet weight concentrations to enable comparisons with our results. Elements not discussed below generally occurred infrequently in eggs and at low concentrations that are not of concern or are known to be relatively non-toxic.

Arsenic - The NOEL and the LOEL for inorganic arsenic reported in eggs were 1.8 and 3.6 µg/g dry weight, respectively or approximately 0.36 and 0.72 µg/g wet weight, respectively. All arsenic concentrations in all years and areas were well below the NOEL.

Boron - The BL, NOEL, and LOEL for boron in eggs were 1.0, 22, and 38 µg/g dry weight, respectively or approximately 0.20, 4.4, and 7.6 µg/g wet weight, respectively. Excluding the Anaho eggs collected in 1988 where the detection limit was high, all boron concentrations were only slightly above the BL and nearly all were an order of magnitude below the NOEL.

Copper - The BL for copper in avian eggs was 5.5 µg/g dry weight, which is approximately 1.1 µg/g wet weight. Copper concentrations in pelican eggs in all years, except 1998 where the detection limit was high, were similar to the BL.

Mercury - The BL, NOEL, and DRT for mercury in avian eggs were reported to be 0.1, 3.0, and 3.0 µg/g dry weight, respectively, which are approximately 0.02, 0.60, and 0.60 µg/g wet weight, respectively. All mercury concentrations exceeded the BL and geometric mean concentrations were somewhat below the NOEL and DRT at Anaho Island in 1988 and 2004 and at Malheur in 1996, with the geometric mean concentrations at Anaho in 1996 being approximately one-third of the NOEL and DRT. Some eggs in all years and locations, except the Ridge sub-colony at Anaho in 1996, had concentrations that exceeded the NOEL and DRT. However, there was no evidence of adverse effects on reproduction at Anaho in relation to mercury concentrations in eggs in 1996 (Wiemeyer et al. 2001).

Molybdenum - The BL, NOEL, and LOEL for molybdenum in avian eggs were reported to be 0.25, 23, and 23 µg/g dry weight, respectively, which are approximately 0.05, 4.6, and 4.6 µg/g wet weight, respectively. Molybdenum concentrations in pelican eggs were generally near the BL when adequate detection limits were used.

Selenium - The BL, NOEL, and DRT for selenium in avian eggs were reported to be 1.9, 6.0, and 6.0 µg/g dry weight respectively, which are approximately 0.38, 1.2, and 1.2 µg/g wet weight, respectively. The geometric mean selenium concentrations in pelican eggs from Anaho Island in 1996 were approximately twice the BL, with eggs from other years and areas having concentrations approximately equal to or lower than the BL. A few eggs from Anaho in 1996 had concentrations that approached, but did not exceed, the NOEL and DRT.

Zinc - The BL for zinc in avian eggs was reported to be 50 µg/g dry weight, which is approximately 10 µg/g wet weight. Geometric mean zinc concentrations in all pelican eggs reported herein were slightly below this level.

Conclusions

Mercury concentrations continue to be of greatest concern due to the elevated levels found in livers of some adult pelicans from the area (Wiemeyer et al. 2001). However, no evidence of adverse effects of mercury on hatching success at the Anaho colony has been found to date. Mercury concentrations in eggs likely fluctuate in response to feeding areas used by the pelicans. However, additional data are needed on feeding areas in response to different wetland conditions. Runoff in the Truckee River also has marked effects on spawning runs and thus the availability of cui-ui to pelicans. Additional data area needed on mercury concentrations in livers of adult and nestling pelicans to determine if concentrations may be having adverse effects on health and survival.

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Table 1. Concentrations (µg/g wet weight) of metals and trace elements and eggshell thickness (mm) of American white pelican eggs from Anaho Island, Nevada and Malheur National Wildlife Refuge, Oregon.

Element or Shell thickness	Anaho Island 1988 (n = 11)	Anaho Island Area C 1996 (n = 15)	Anaho Island Ridge 1996 (n = 15)	Anaho Island Bluff 2004 (n = 10)	Malheur 1996 (n = 6)
Aluminum	nd[a] (0)[b] [<9.5]	0.93 (14) [<0.83-1.14][c]	nd (6) [<0.79-0.92]	0.29 (6) [<0.26-0.69]	0.80 (5) [<0.79-1.07]
Arsenic	0.011 (10) [<0.005-0.03]	nd (1) [<0.07-0.10]	nd (0) [<0.08-<0.09]	nd (0) [<0.03-<0.04]	nd (0) [<0.07-<0.08]
Barium	nd (0) [<4.8]	nd (3) [<0.07-0.24]	nd (3) [<0.08-0.09]	0.17 (10) [0.10-0.29]	nd (2) [<0.07-0.23]
Boron	nd (0) [<4.8]	nd (7) [<0.27-0.51]	nd (7) [<0.31-0.49]	nd (2) [<0.26-0.35]	nd (0) [<0.32-<0.37]
Chromium	nd (0) [<0.95]	0.10 (10) [<0.07-0.47]	nd (0) [<0.08-<0.09]	nd (0) [<0.07-<0.09]	nd (0) [<0.07-<0.08]
Copper	nd (1) [<2.4-3.09]	0.99A[d] (15) [0.89-1.15]	0.97A (15) [0.79-1.19]	1.05A (10) [0.95-1.23]	1.00A (6) [0.94-1.08]
Iron	15.5A (10) [<9.5-24]	17.1A (15) [13-24]	16.3A (15) [13-22]	17.4A (10) [13-22]	16.6A (6) [13-21]
Mercury	0.47B (11) [0.24-0.72]	0.17A (15) [0.03-1.07]	0.19A (15) [0.07-0.55]	0.39AB (10) [0.14-0.86]	0.36AB (6) [0.20-1.05]
Magnesium	nd (3) [<95-101]	98.AB (15) [81-124]	90.A (15) [71-106]	106.B (10) [95-122]	90.A (6) [84-98]
Manganese	nd (0) [<1.4]	0.21AB (15) [0.17-0.34]	0.22B (15) [0.14-0.30]	0.20AB (10) [0.17-0.30]	0.15A (6) [0.10-0.32]
Molybdenum	nd (0) [<4.8]	0.08 (11) [<0.07-0.13]	nd (4) [<0.08-0.10]	nd (0) [<0.26-<0.35]	nd (0) [<0.07-<0.08]

Table 1. continued

Element or Shell thickness	Anaho Island 1988 (n = 11)	Anaho Island Area C 1996 (n = 15)	Anaho Island Ridge 1996 (n = 15)	Anaho Island Bluff 2004 (n = 10)	Malheur 1996 (n = 6)
Nickel	nd (0) [<3.7]	0.08 (8) [<0.07-0.31]	nd (0) [<0.08-<0.09]	nd (0) [<0.07-<0.09]	nd (0) [<0.07-<0.08]
Selenium	0.20A (9) [<0.10-0.54]	0.74C (15) [0.34-1.18]	0.72C (15) [0.45-1.02]	0.26AB (10) [0.19-0.50]	0.40BC (6) [0.32-0.47]
Strontium	0.80A (6) [<0.95-1.74]	1.91B (15) [1.0-3.9]	1.35B (15) [1.1-1.9]	1.33B (10) [1.1-1.9]	0.62A (6) [0.39-0.90]
Zinc	6.5A (10) [<1.8-10.7]	7.5A (15) [6.4-9.0]	6.6A (15) [5.4-7.9]	7.3A (10) [6.7-8.6]	5.9A (6) [5.1-7.2]
Shell thickness	NA	0.662	0.622	0.642	0.661
Percent change		-2.9	-8.8	-5.9	-3.1

[a] Geometric mean; nd = not computed, < 50% with detectable residues

[b] Number with detectable residues in parentheses.

[c] Extremes in brackets.

[d] Means for a given contaminant followed by a common capital letter are not significantly different ($p > 0.05$; Bonferroni multiple comparison test).

[e] Arithmetic means reported for shell thickness. NA = data not available. Percent change based on pre-DDT norm of 0.682 mm (D. Anderson, pers. comm.).