LA-UR-04-0991 **February 2004**

Approved for public release; distribution is unlimited.

Title

Contaminants in Eggs of Western Bluebirds and Ash-Throated Flycatchers at Los Alamos National Laboratory, New Mexico

Authors

Jeanne M. Fair
Charles D. Hathcock
Kaia L. Colestock

RRES-Ecology Group, MS M887
Los Alamos National Laboratory
Los Alamos, NM 87545
Telephone: 505-667-5659
Fax: 505-667-0731
E-mail: jmfair@lanl.gov

Los Alamos
NATIONAL LABORATORY
Los Alamos, New Mexico 87545

*An Affirmative Action/Equal Opportunity Employe*r

Abstract

Eggshell quality, clutch size, sex ratio, and hatching success of western bluebirds (*Sialia mexicana*) and ash-throated flycatchers (*Myiarchus cinerascens*) were studied on a landscape-soil contaminant gradient at Los Alamos National Laboratory (LANL) in New Mexico from 1997-1999. A variety of contaminants (heavy metals, chemicals, insecticides, polychlorinated biphenyls, organochlorines, and radioactive isotopes) range across different spatial scales and concentrations on LANL land. This study is an example of a monitoring program over a large area with varying degree of contamination that is used to highlight locations of concern for future research. There were two locations where the flycatcher had a lower hatching success. The bluebirds at Sandia wetland, a location of concern for poly-chlorinated biphenyls (PCBs), had a thinner eggshell thickness index (RATCLIFFE) and the eggs were smaller than at other locations. The flycatcher had thinner eggshells than bluebirds, which could add to sensitivity to exposure to contaminants. There was no variation in clutch size or sex ratio between locations or areas closer to contaminant release sites for both species. Percent females in the clutch ranged from 0-100 % in the WEBL and 33-67 % for ATFL.

***Key Words*:** Western bluebird; ash-throated flycatcher; PCBs; radionuclides; organochorines

INTRODUCTION

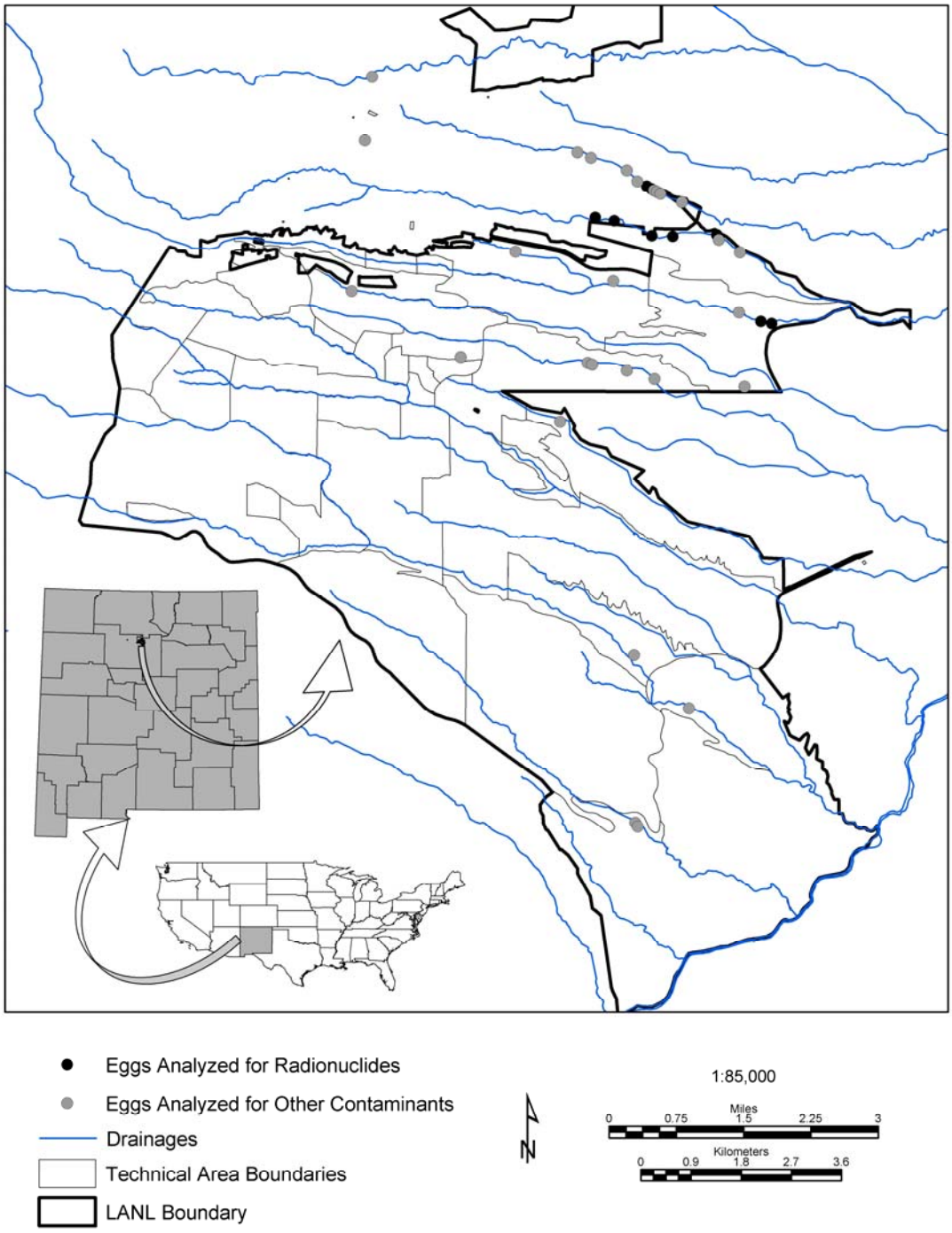
Understanding how chronic exposure to soil contamination affects both individuals and populations is crucial for remediation efforts. However, this can be a difficult challenge when the contamination is uncharacterized and contains a mixture of contaminants including chemicals, radionuclides, and heavy metals. Understanding both exposure response relationships are key to (1) assessing the implications for the exposure of management endpoints, (2) designing and implementing effective remediation strategies, and (3) for documenting post-remediation recovery. A complementary approach to estimating the impacts of contaminants will include both the collection of biological meaningful data on the impacts on health and condition to life history traits such as clutch size and sex ratio, and exposure data actual bioavailable concentrations. Inherent in this approach would be to develop a monitoring network of several species across the gradient of contaminated areas, focusing on individual life-history traits that can have population–level impacts and the availability of eggs for contaminant concentrations.

There are several good reasons for using eggs rather than adult animals for contaminant residue studies. The first is that it is far less destructive for the population, especially if nonviable eggs are used. The second reason is that this life stage is often the most sensitive to contaminants and therefore critical for the understanding of impacts to a species (Peakall D.B. 1994). No only do female birds have the advantage of the ability of elimination of contamination in eggs, juveniles also have the advantage of a rapid elimination of contaminants that shuts down as they age (Fair et al. 1994).

This study was conducted at Los Alamos National Laboratory (LANL) in northern New Mexico (Figure 1). Many activities and operations at the Laboratory have used or produced liquids, solids, and gases that contain radioactive or nonradioactive hazardous materials. A variety of contaminants (heavy metals, chemicals, insecticides, PCBs, polyhalogenated hydrocarbons, organocholrines, and radioactive isotopes) range across different spatial scales and soil concentrations on LANL land. Potentially contaminated sites are located in a clumped distribution across the landscape at LANL, with no overall specific contaminant that is inherently everywhere. Wildlife at LANL may be exposed to various mixtures of contaminants that could affect animal populations by reducing reproduction or survival. It not only crucial to have information that is biologically relevant (i.e. able to be extrapolated to meaningful effects) concerning ecological risks, but to have exposure estimates of contaminants that are bioaccessible to migratory birds as well.

The western bluebird (WEBL) is a widely distributed, sexually dichromatic, and monogamous species. The ash-throated flycatcher (ATFL) is not as widely distributed or sexually dichromatic. Both species nest in secondary nest cavities, are insectivorous during the breeding season, and use small amounts of grit in their gizzards that are potentially important exposure pathways. These two species have similar life history traits, although the ATFL has a faster rate of development, fledges 4-5 days earlier than the bluebird, and has a significantly higher field metabolic rate during development (Mock et al., 1991). This difference in duration of development period could affect the

Figure 1. Los Alamos National Laboratory and box locations where western bluebird and ash-throated flycatcher eggs were collected from 1997 to 2003.



relative exposure and risks to contaminants. If intake of contaminants in soil is proportional to dry matter intake as is assumed in ecological risk methodology, the higher metabolic rate for the ATFL compared to the WEBL may increase their relative risk of toxic exposure. Both species feed on similar prey items on and above the ground. Sexual dichromatisim differs in the two species, with the WEBL being sexually dichromatic and the ATFL having no sexual differences. Both bird species in this study readily utilized nestboxes and are common in northern New Mexico.

The objectives of this study were to pinpoint areas of concern for environmental restoration at LANL for two sympatric avian species with similar life-history traits and to report on organochorline, heavy metal, polycyclic aromatic hydrocarbons, and radionuclide concentrations in two passerine species eggs collected from various locations at LANL.

MATERIALS AND METHODS

Study sites and field methods

The 111 km^2 Los Alamos National Laboratory is situated on the Pajarito Plateau and consists of a series of relatively narrow mesas separated by deep, steep-sided canyons that decline east-southeast from the Jemez Mountains down to the Rio Grande River. Six major vegetation community types are found in Los Alamos County: subalpine grassland, spruce–fir forest, mixed conifer forest, ponderosa pine forest, piñon–juniper woodland, and juniper grasslands (Foxx and Tierney, 1985). In general, Los Alamos has a temperate montane climate with four distinct seasons. Annual precipitation is 47.6 cm.

During the winter of 1997, 438 nestboxes were placed on LANL in total of 18 both potentially contaminated and reference areas. Nestboxes were placed

approximately two meters off the ground on trees and spaced approximately 50-75 meters apart. Boxes were placed in the open ponderosa pine forest of the canyons and piñon–juniper woodland on the plateau mesas. Boxes were placed in 18 locations or areas on LANL land with an average of 29 boxes per location.

Starting in May 1997, nestboxes were visited and nests with eggs were considered active and visited every two days until the first eggs hatched (day = 0). The animal care and use committees of both LANL and the University of Missouri-St. Louis approved all protocols. Data were collected for the summer breeding seasons of 1997 to 2003.

Egg sampling

Unhatched eggs were collected from the nestboxes when the nestlings were past the age of 10 days old. Eggs were stored in a refrigerator until measurements were taken (1-10 weeks). Egg volume was measured by water displacement and greatest length and breadth were measured for each egg. Eggshells were opened, rinsed, and dried. All dirt, uric acid, or other materials were cleaned away. The contents were stored at –30° C for the residue analysis.

Analysis of egg contaminant concentrations

The target analyte list included radioisotopes, heavy metals, organochlorines, polyaromatic biphenyls, heavy metals, and polycyclic aromatic hydrocarbons and listed in Table 1. The radioisotopes analyzed included Americium-241, Cesium-137, Plutonium-238, Plutonium-239, and Strontium-90.

Table 1. Target contaminant analyte list for residue analysis in avian eggs from Los Alamos, New Mexico 1997-2003.

	Pesticides/Herbecides		CAS#	PCBs		CAS#	PCBs		Metals		PAHs
1	α--BHC	1	5	2,3-Dichlorobiphenyl	16	95	2,2',3,5',6-Pentachlorobiphenyl[(a)]	1	Chromium	1	Acenaphthene
2	β -BHC	2	8	2,4'-Dichlorobiphenyl	17	99	2,2',4,4',5-Pentachlorobiphenyl	2	Manganese	2	Acenaphthylene
3	α -Chlordane	3	18	2,2',5-Trichlorobiphenyl	18	105	2,3,3',4,4'-Pentachlorobiphenyl	3	Nickel	3	Anthracene
4	γ--Chlordane	4	28	2,4',4-Trichlorobiphenyl	19	110	2,3,3',4',6-Pentachlorobiphenyl[(a)]	4	Copper	4	Benzo(a)anthracene
5	4,4'-DDD	5	31	2,4',5-Trichlorobiphenyl	20	118	2,3',4,4',5-Pentachlorobiphenyl[(a)]	5	Zinc	5	Chrysene
6	4,4'-DDE	6	33	2',3,4-Trichlorobiphenyl	21	128	2,2',3,3',4,4'-Hexachlorobiphenyl	6	Arsenic	6	Benzo(b)fluoranthene
7	4,4'-DDT	7	44	2,2',3,5'-Tetrachlorobiphenyl	22	138	2,2',3,4,4',5'-Hexachlorobiphenyl	7	Selenium	7	Benzo(k)fluoranthene
8	Dieldrin	8	49	2,2',4,5'-Tetrachlorobiphenyl	23	163	2,3,3',4',5,6-Hexachlorobiphenyl	8	Silver	8	Fluoranthene
9	Heptachlor	9	52	2,2',5,5'-Tetrachlorobiphenyl	24	149	2,2',3,4',5',6-Hexachlorobiphenyl[(a)]	9	Cadmium	9	Fluorene
10	Lindane	10	66	2,3',4,4'-Tetrachlorobiphenyl[(a)]	25	153	2,2',4,4',5,5'-Hexachlorobiphenyl	10	Antimony	10	Phenanthrene
11	Methoxychlor	11	70	2,3',4',5-Tetrachlorobiphenyl	26	180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	11	Barium	11	Pyrene
12	t-nonachlor	12	74	2,4,4',5-Tetrachlorobiphenyl	27	183	2,2',3,4,4',5',6-Heptachlorobiphenyl	12	Lead	12	Benzo(a)pyrene
13	HPX + OXC (by GC - ECD)	13	77	3,3',4,4'-Tetrachlorobiphenyl[(a)]	28	187	2,2',3,4',5,5',6-Heptachlorobiphenyl	13	Mercury	13	Benzo(g,h,l,)perylene
14	HPX (by difference)	14	84	2,2',3,3',6-Pentachlorobiphenyl	29	194	2,2',3,3',4,4',5,5'-Octochlorobiphenyl			14	Dibenzo(a,h)anthracene
15	OXC (by GC / MS)	15	101	2,2',4,5,5'-Pentachlorobiphenyl	30	201	2,2',3,3',4,5',6,6'-Octochlorobiphenyl			15	Indeno(1,2,3-cd)perylene

Radioisotopes

Polyaromatic biphenyls, organochlorines, and polycyclic aromatic hydrocarbons

The Illinois Waste Management and Research Center completed analyses of the egg tissue samples for metals, polyaromatic biphenyls (PCB), Organochlorines (OC), and polycyclic aromatic hydrocarbons (PAH). Upon receipt, the egg samples were stored in a freezer maintained at -60^{o} C. As needed, the samples were removed from the freezer, and, after thawing, mixed with an aliquot of sodium sulfate. The sample mixtures were extracted with a Soxtec apparatus (simulates soxhlet extraction) and then cleaned up with gel permeation chromatography (GPC) and silica gel chromatography. Samples were homogenized prior to digestion with a mortar and pestle. A nitric acid microwave digestion procedure, equivalent to US EPA Method 3051, was utilized to dissolve egg tissues into solution for total metals analysis. In addition, quality control samples were prepared with your tissues in each of 5 separate digestion batches. Quality control samples with each batch included a digestion reagent blank, a duplicate sample, a matrix spike, and a standard reference material.

The samples were analyzed for individual PCB congeners and OC compounds by gas chromatography utilizing an electron capture detector. The numbers assigned to the individual congeners follows the Balschmiter and Zell protocol. An exception occurs with congener 200/201 where the 200 is the BZ# and the 201 is the IUPAC designation. Whenever a PCB or OC was detected, the sample was reinjected on a gas chromatography/mass spectrometry (GC/MS) instrument to confirm the findings. The PAHs were analyzed by GC/MS only.

Heavy metals

Results for metals were obtained by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using Scandium, Yttrium, Rhodium, and Thorium as internal standards. Results for Mercury were obtained by Atomic Fluorescence (A P S Analytical Mercury Atomic Fluorescence Analytical System).

The sample is pumped into the nebulizer chamber of the ICPMS through small diameter tubing by a peristaltic pump. In the nebulizer chamber, the liquid sample stream flows over an argon gas stream and is entrained in it as a fine aerosol mist. The sample-saturated argon is then aspirated into the torch. The plasma torch is sufficiently energetic enough (6000 – 8000 K) to desolvate the mobile phase droplets into dry particles, atomize the dry particles (provided the particles are less than approximately 10 μm in diameter), and ionize the elements of interest in the amount of time (a few milliseconds) that it takes for the droplets/particles to traverse the length of the torch. The plasma torch is generated by passing Argon gas through the center of a load coil through which a high alternating current (AC) is passed. The AC current, operated at radio frequencies (rf), induces a strong alternating magnetic field in the Argon gas. An initial direct current (DC) electrical discharge though the gas causes some Argon ions to form. The powerful alternating magnetic field then causes the few gas ions to rapidly move back and forth where they collide with other Argon atoms. The ions strike other atoms with sufficient energy that they cause the loss of a electron and in the process create more ions and heat which is the basis for the plasma. Thus the rf current sustains the plasma and provides the source of energy required to ionize the analytes of interest.

Each ion striking the detector creates a pulse of electric current. Pulses for each m/z are counted and summed as a count rate or intensity. The greater the concentration of

analyte in the original solution, the greater the number of pulses counted over a constant time interval, hence the greater the intensity. The count rate for analytes of interest in samples is fed back to the computer, where it is converted to a concentration using an intensity-to-concentration calibration curve obtained on standards.

Statistical analysis

The statistical techniques used followed the guidelines for statistical analysis radiological monitoring in the Regulatory Guide for Radiological Effluent Monitoring and Environmental Surveillance of the U.S. Department of Energy (DOE) (DOE, 1991).

Monitoring programs often include measurement of extremely low concentrations of radionuclides, below the detection limit of the counting instruments (DOE, 1991). All of the radionuclides in the eggs were at less-than-detectable measurements. In concordance with the DOE guidelines (DOE 1991), all of the actual values, including those that were negative, were included in the statistical analysis. Practices such as assigning a zero, the detection limit value, or some in-between value can severely bias the resulting parameters estimates and should be avoided. While it important to present that all values are below the detection limits for each radionuclide in this study, differences between species, year, and locations were analyzed. Radionuclides were transformed to a lognormal distribution.

The Statistical Analysis System (SAS, Institute, Inc., 1987) was used for all statistical analyses, and assumptions for parametric statistics were examined. Analysis of covariance was used for all comparisons between locations and potentially contaminated areas (PROC GLM). Means for comparison group were compared with

Duncan's Multiple Range Test. Data not normally distributed or having heteroscedastic variances were compared with Kruskal-Wallis nonparametric tests. Eggshell thickness was compared between species using egg volume as a covariate to control for thickness being correlated with egg size. Elevation was also used as a covariate in all Analysis of Variance (ANOVA) models.

All nestbox locations were obtained using a non-differentially corrected GPS (Garmin GPS III Personal Navigator™, Olathe, KS, USA) with real–time FM correction. Locations were checked for accuracy, and contaminant data were accessed using ArcView© (ESRI, 1996).

RESULTS

Radio isotopes

Organochlorines

Polyaromatic biphenyls

Heavy metals

Polycyclic aromatic hydrocarbons

DISCUSSION

In the first three years of this study, western bluebirds and ash-throated flycatchers had similar hatching success and hatching success was not correlated with elevation, Julian hatch date, distance to nearest potential contaminated location (Fair and Myers 2002). There was no variation in percent hatching among the different areas for the WEBL, but in two areas ATFL had below average hatching success (Technical Area 33 and Los Alamos Canyon).

The ATFL eggs were longer than the WEBL eggs ($F_{1, 94} = 7.19$, $p = 0.009$). Western bluebirds had a much thicker shell than the ash-throated flycatcher. There appears to be more variation with the eggshell index for the bluebirds in regards to location, ranging from 11.3 to 18.2 ($F_{6, 72} = 3.33$, $p = 0.007$). Sandia wetland contains eggs with an average eggshell index 9% thinner than the other six locations with more than four eggs collected (Duncan's Multiple Range Test).

Clutch size for both species also did not vary with the type of PRS or between potentially contaminated sites and sites farther away.

One category of contaminants of concern for eggshell thinning is the PCBs (Frumkin, 1994). Numerous studies have investigated the effects of chlorinated hydrocarbons on eggshells (Ratcliffe, 1967; Hickey and Anderson, 1968; Anderson and Hickey, 1970; Blus et al 1972; Cooke 1973; Morrison and Kiff 1979; Weimeyer et al., 1984; Lundholm, 1987; Fair et al., 1994). Fernie et al. (2000) also found that water content and eggshell thickness were not affected by PCB exposure but that yolks in the PCB-contaminated eggs were heavier and suggest that the contaminated eggs have relatively more lipid and less protein available for embryonic development.

Two other important factors involved in shell thickness may be prior exposure to DDT on wintering grounds and taxon specific response to DDT. The ATFLs migrate south from New Mexico, although it is not known specifically where they migrate. Mexico and several countries in Central America continue to use DDT pesticides that could be an exposure route to the migratory birds from North America. Banded WEBLs in this study were spotted throughout the winters on the Pajarito Plateau. Western bluebirds in the southwestern part of North America may only migrate in colder and moister years (J. Guinan, personal communication).

The WEBL eggs from nests in Sandia wetland were smaller and had a thinner eggshell index than other WEBL eggs from other areas. Many nonexperimental studies have reported a positive relationship between egg size and posthatching survival or growth in birds, and few experimental studies have demonstrated a developmental advantage of larger eggs (Bolton, 1991; Hipfner and Gaston, 1999). While in some species, parental quality can contribute as much as egg size (Blomqvist et al., 1997); areas consistently producing small eggs could indicate poor habitat.

The hatching success of these two species was lower and much more variable than reported for two similar cavity-nesting birds by Eeva and Lehikoinen (1995). They reported markedly lower hatching success closer to a factory complex producing heavy metal pollutants and less variation in hatching success farther away. Eeva and Lehikoinen (1995) also suggested that hatching failure did not result from lower fertility of the eggs, but rather from desiccation of eggs with low-quality shells from exposure to contaminants.

There was not the seasonal shift in nestling sex ratios of the earlier part of the season being biased towards males and later in the season biased towards females, as found by Smallwood and Smallwood (1998) for American kestrels (*Falco sparverius*). Several reported incidences of skewed sex ratios in reptiles have been reportedly caused by xenobiotics such as DDT, polychlorobiphenyls (PCBs), and tertacholor dibenzodioxin (TCDD), which are referred to as endocrine disrupters (Guillette et al., 1995). Miyamoto and Klein (1998) warn that effects from these contaminants should not be considered broad and general, due to species differences. Passerines have been found to have female biased sex ratios (Gowaty 1993) and this may add difficulty in the interpretation, sex ratio investigations within a species can be used in varying environments.

The varying results of the sensitivities of the different life-history traits of different species suggest that consideration of life history may be more important for site-specific assessments. If potential hazards to anthropogenic stressors and safe environmental contaminant concentrations are to be estimated, then a life-cycle approach using numerous life-history traits for each stage is critical. The next logical step is a sensitivity analysis of population dynamics of these two species, elucidating which life-history traits are sensitive. Questions such as the importance of a clutch size decrease to population growth can be answered. Viability analyses are lacking for large or apparently healthy passerine populations.

Acknowledgements

We thank the following people for excellence in field and laboratory assistance: K. Colestock, J. Fox, L. Haussamen, V. Mason, and S. Whitaker. This research was

funded by the US Department of Energy contract no. W-7405-ENG-36 to Los Alamos National Laboratory and the LANL Environmental Restoration Project.

References

(ESRI) Environmental Science Research Institute, 1996. ArcView (version 3.2 UNIX), GIS, USA.

Anderson, D.W., Hickey, J.J., 1970. Oological data on egg and breeding characteristics of brown pelicans. Wilson Bulletin 82, 14-28.

Bennett, K., Keller, D., Robinson, R., 2001. Sandia Wetland Evaluation. Los Alamos National Laboratory Report, LA-UR-01-66.

Bennett, K., Biggs, J., Gonzales, G., 1999. Evaluation of PCB concentrations in small mammals in Sandia Canyon. Los Alamos National Laboratory Report, LA-99-5891.

Blomqvist, D., Johansson, O.C., Gotmark, F., 1997. Parental quality and egg size affect chick survival in a precocial bird, the lapwing, *Vanellus vanellus.* Oecologia 110, 18-24.

Blus, L. J., Gish, C.D., Belisle, A.A., Prouty, R.M., 1972. Logarithmic relationship of DDE residues to eggshell thinning. Nature 235,376-377.

Bolton, M., 1991. Determinants of chick survival in the lesser black-backed gull: relative contributions of egg size and parental quality. Journal of Animal Ecology 60, 949-960.

Brook, B.W., Burgman, M.A., Frankham, R., 2000. Differences and congruencies between PVA packages: the importance of sex ratio for predictions of extinction risk. Conservation Ecology 4,6. (online) URL: http://www.consecol.org/vo14/iss1/art6

Brooker, M.G., Brooker, L.C., 1991. Eggshell strength in cuckoos and cowbirds. Ibis 133, 406-413.

Cooke, A.S., 1973. Shell thinning in avian eggs by environmental pollutants. Environmental Pollution 4, 85-152.

Donaldson, G. M. & Braun, B.M., 1999. Sex-related levels of selenium, heavy metals, and organochlorine compounds in American white pelicans (*Pelecanus erythrorrhyncos*). Archives Environmental Contamination and Toxicology 37, 110-114.

Eeva, T., Ojanen, M., Rasanen, O., Lehikoinen, E., 2000. Empty nests in the great tit (*Parus major*) and the pied flycatcher (*Ficedula hypoleuca*) in a polluted area. Environmental Pollution 109, 303-309.

Eeva, T., Lehikoinen, E., 1995. Egg shell quality, clutch size and hatching success of the great tit (*Parus major*) and the pied flycatcher (*Ficedula hypoleuca*) in an air pollution gradient. Oecologia 102, 312-323.

Fair, J.M. 2000. The ecological and physiological costs of contaminants on birds: consequences for individuals and populations. Ph.D. Dissertation, University Missouri-St. Louis.

Fair, J. M., McEwen, L.C., Kennedy, P.L., 1994. Organochlorine residues in nesting killdeer, (*Charadrius vociferus*) and their eggs in North Dakota. Canadian Field Naturalist 108, 328-331.

Fernie, K.J., Bortolotti, G.R., Smits, J.E.,Wilson, J., Drouillard, K.G., Bird, D.M., 2000. Changes in egg composition of American kestrels exposed to dietary

polychlorinated biphenyls. Journal of Toxicology and Environmental Health Part A. 60, 291-303.

Fitzgerald, L.A., 1994. The interplay between life history and environmental stochasticity: implications for the management of exploited lizard populations. American Zoologist 34, 371-381.

Foxx, T.S., Tierny, G.D., 1980. Status of the flora of the Los Alamos National Environmental Research Park. Los Alamos National Laboratory, Los Alamos, NM.

Frumkin, R., 1994. Does PCB reduce shell thickness and strength in sparrowhawk eggs? Journal Fur Ornithologie 135, 527.

Gaunt, A. S. & Oring, L.W., 1997. Guidelines to the use of wild birds in research. The Ornithological Council, Washington, DC.

Glooseschenko, V.B., Herskowitz, J., Fulthorpe, R., Rang, S., 1986. Association of wetland acidity with reproductive parameters and insect prey of the eastern kingbird (*Tyrannus tyrannus*) near Sudbury, Ontario. Water Air Soil Pollution 30, 553-567.

Gowaty, P.A. 1993. Differential dispersal, local resource competition, and sex ratio variation in birds. American Naturalist 141, 263-280.

Grandjean, P., 1976. Possible effects of lead on egg-shell thickness in kestrels. Bulletin of Environmental Contamination and Toxicology 16, 101-106.

Graveland, J., Drent, R.H., 1997. Calcium availability limits breeding success of passerines. Journal of Animal Ecology. 66, 279-288.

Graveland, J., Van Der Wal, R., Balen, J.H. Van, Noordwijk, A.J. Van., 1994. Poor reproduction in forest passerines from decline of snail abundance on acidified soils. Nature 368, 446-448.

Griffiths, R. D., S., and Dijkatra, C., 1996. Sex identification in birds using two CHD genes. Proceeding of the Royal Society of London Series B 263, 1251-1256.

Hickey, J.J., Anderson, D.W., 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. Science 162, 271-273.

Hipfner, J. M., Gaston, A.J., 1999. The relationship between egg size and posthatching development in the thick-billed murre. Ecology 80, 1289-1297.

Johnson, L. S., Barclay, M.R. 1996. Effects of supplemental calcium on the reproductive output of a small passerine bird, the house wren (*Troglodytes aedon*). Canadian Journal of Zoology 74, 278-282.

Landis, W. G. & Yu, M.H., 1995. Introduction to environmental toxicology : impacts of chemicals upon ecological systems. Lewis Publishers, Boca Raton.

Longmire, P.A., Reneau, S.L., Watt, P.M., McFadden, L.D., Gardner, J.N., Duffy, C.J., Ryti, R.T., 1996. Natural background geochemistry, geomorphology, and pedogenesis of selected soil profiles and Bandelier Tuff Los Alamos, New Mexico. Los Alamos National Laboratory Report LA-12913-MS.

Lundholm, E., 1987. Thinning of eggshells in birds by DDE: mode of action on the eggshell gland. Comparative Biochemistry and Physiology 88C, 1-22.

Miles A.K., G., C.E., Pendleton, G.W., Soares, J.H., Jr., 1993. Effects of dietary aluminum, calcium, and phosphorus on egg and bone of European starlings. Archives of Contamination and Toxicology 24, 206-212.

Miyamoto, J., Klein, W., 1998. Environmental exposure, species differences and risk assessment. Pure Applied Chemistry 70,1829-1845.

Mock, P.J., Khubesrian, M., Larcheveque, D.M., 1991. Energetics of growth and maturation in sympatric passerines that fledge at different ages. Auk 108, 34-41.

Morrison, M.L., Kiff L.F. 1979. Eggshell thickness in American shorebirds before and since DDT. Canadian Field Naturalist 95, 187-190.

Myers, O.B.,1999. On aggregating species for risk assessment. Human and Ecological Risk Assessment 5, 559-574.

Nager, R.G., Monaghan, P., Griffiths, R., Houston, D.C. Dawson, R., 1999. Experimental demonstration that offspring sex ratio varies with maternal condition. Proceedings of the National Academy of Science 96, 570-573.

Ormerod, S.J., Bull, K.R., Gummins, C.P., Tyler, S.J., Cickery, J.A., 1988. Egg mass and shell thickness in dippers *Cinclus cinclus* in relation to stream acidity in Wales and Scotland. Environmental Pollution 55, 107-121.

Ormerod, S.J., S.J. Tyler, and I. Jüttner., 2000. Effects of point-source PCB contamination on breeding performance and post-fledging survival in the dipper *Cinclus cinclus*. Environmental Pollution 109, 505-513.

Ratcliffe, D.A., 1967. Decrease in eggshell weights in certain birds of prey. Nature 215, 208-210.

Risebrough, R.W., 1999. 'Endocrine disruption' and the 'wildlife connection'. Human and Ecological Risk Assessment 5, 869-883.

Rothstein, S.I., 1972. Eggshell thickness and its variation in the cedar waxwing. Wilson Bulletin 84, 469-473.

SAS Institute, Inc., 1987. SAS/STAT guide for personal computers. SAS Institute, Inc, Cary, NC.

Scheuhammer, A.M., 1996. Influence of reduced dietary calcium on the accumulation and effects of lead, cadmium, and aluminum in birds. Environmental Pollution 94, 337-343.

Sheldon, B.C., Merila, J., Lindgren, G., Ellegren, H., 1998. Gender and environmental sensitivity in nestling collared flycatchers. Ecology 79, 1939-1948.

Siikamaki, P. 1998. Limitation of reproductive success by food availability and breeding time in pied flycatchers. Ecology 79, 1789-1796.

Smallwood, P.M., Smallwood, J.A., 1998. Seasonal shifts in sex ratios of fledgling American kestrels (*Falco sparverius paulus*): the early bird hypothesis. Evolutionary Ecology 12, 839-853.

Weimer, V., Schmidt, K.H., 1998. Untersuchungen zur eiqualitat bei der kohlmeise (*Parus major*) in abhangigkeit von der bodenbeschaffanheit. Journal of Ornithology 139, 3-9.

Wiemeyer, S.N., Lamont, T.G., Bunck, C.M., Sindelar, C.R., Gramlich, F.J., Fraser, J.D., Byrd, M.A., 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs-1969-79-and their relationships to shell thinning and reproduction. Archives Environmental Contamination and Toxicology 13, 529-549.

APPENDICES

Figure 1. Nestbox locations in canyons and mesas at Los Alamos National Laboratory.

Figure 2. Percentage of hatching for western bluebirds and ash-throated flycatchers for 1997-1999.

Figure 3. Julian hatch date for western bluebirds and ash-throated flycatchers for 1997-1999

Figure 4. Clutch size for western bluebirds and ash-throated flycatchers for 1997-1999

Figure 5. Percentage of females for western bluebirds and ash-throated flycatchers for 1997-1999