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Los Alamos NATIONAL LABORATORY

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Avian Individual and Population Health on Los Alamos National Laboratory, 2001 Update

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Abstract

In 1997 an avian monitoring system was established in Mortandad Canyon and other areas of concern as well as reference areas at Los Alamos National Laboratory. The purpose of the investigation was to evaluate the health and condition of individuals and populations in areas of concern for potential exposure to contaminants. Since 1963, treated radiological liquid effluent was discharged into the upper reaches of Mortandad, from which it flows down the canyon until it sinks into the alluvium. From 1997 to 1999 physiological markers that included immunocompetence, hematology, growth, and behavior assessed the individual health of the birds. Parameters that were investigated in 2001 included nest productivity and survival. All adult western bluebirds that could be captured were banded. This capture and recapture data will be included in a population viability analysis that includes data from long-term bluebird populations in Arizona, Oregon, and California. Overall, breeding pairs of western bluebirds was down significantly from pre-Cerro Grande fires numbers. Birds breeding in Mortandad canyon have a slightly higher probability of survival than birds breeding in other locations of LANL and this may be due to the habitat of this open canyon with a diversity of edge environments. This also may be evidenced in the high occupancy rates of boxes in Mortandad canyon after the Cerro Grande fire where birds are opportunistically selecting this canyon for breeding. It is clear from this research, that Mortandad canyon is an important breeding area for cavity nesting birds on the Pajarito Plateau. Due to this importance compared to other locations at LANL, Mortandad canyon should continue be monitored in the future. Contaminant concentration exposure assessment would be valuable in determining the potential impacts to passerines in this canyon.

Introduction

In 1997, an avian nestbox monitoring network was established on LANL to investigate the health and condition of cavity-nesting bird populations on the Pajarito Plateau (Fig. 1). Because life-history traits directly affect reproduction and survival, they direct our attention to factors that bear on the extinction rates of plant and animal populations. Understanding life-history traits and how they shape the population is critical not only for rare species but also for outbreak and exotic species that jeopardizes the existence of native or endemic species. Life-history theory shows quantitatively how stress on organisms affects fitness (Sibly and Calow 1989). The concept of maximizing fitness is the key to insight into the demographic consequences of toxic effects at the individual level (Sibly and Calow 1989). Integrating ecological theory and understanding of ecological processes should be the first step in all ecological risk assessments (Calow et al. 1997).

The purpose of this study is to evaluate the magnitude and sources of ecological risks from contaminants for cavity-nesting birds at Los Alamos National Laboratory (LANL) in New Mexico. Ecological risk assessment can be defined as the process of defining and quantifying risks to nonhuman biota and determining the acceptability of those risks (Suter 1993). The main objective is to evaluate the ecological and physiological costs of exposure to various contaminants at LANL, specifically lead shot, and their potential impact on population processes. During the past two decades it has become increasingly

important to be able to predict risks from potential adverse effects of exposure to chemical and physical hazards in the environment. This has resulted in the critical need for estimates of the relationship between exposure of organisms to contaminants and the response of the population.

The first step of this project was the completion of an investigation of individual life history traits that include nestling growth, immunocompetence, and hematology. This first step gave insight into three locations, DP canyon that had lowered immune development, Mortandad canyon and a smaller growth rate, and Sandia wetland that had smaller and thinner eggs. Sandia wetland will be used in a stable isotope study to investigate the uptake and flow of PCBs in the trophic systems. Mortandad canyon which was heavily burnt in the Cerro Grande fire had the highest occupancy of nesting birds than any other area at LANL in 2001.

A wide range of contaminants was released on numerous areas at LANL as a byproduct of various chemical and technological research programs. These contaminants (heavy metals, chemicals, PCBs, and radioactive isotopes) range across different spatial scales and soil concentrations on LANL land. Many of the contaminants at LANL are long-lived (e.g., the half-life of plutonium is 24,000 years). Since LANL is surrounded by undisturbed habitat, this variable landscape provides a perfect opportunity for designing and testing models of ecological risk assessment on wild species.

Cerro Grande Fire, 2000

In May, 2000 the Cerro Grande Fire burned a total of 47,650 acres around Los Alamos with many acres on LANL land (Fig.2). This fire affected much of the habitat for breeding birds on the Pajarito Plateau and also offers the opportunity of comparing pre and post fire breeding bird health, condition and use of these land and investigating the increase in bioavailability of contaminants by the fire. Nyhom (1996) points out that insectivorous birds continue to be an extremely useful monitor of pollution and management practices. Mortandad canyon completely lost 51 of 55 boxes.

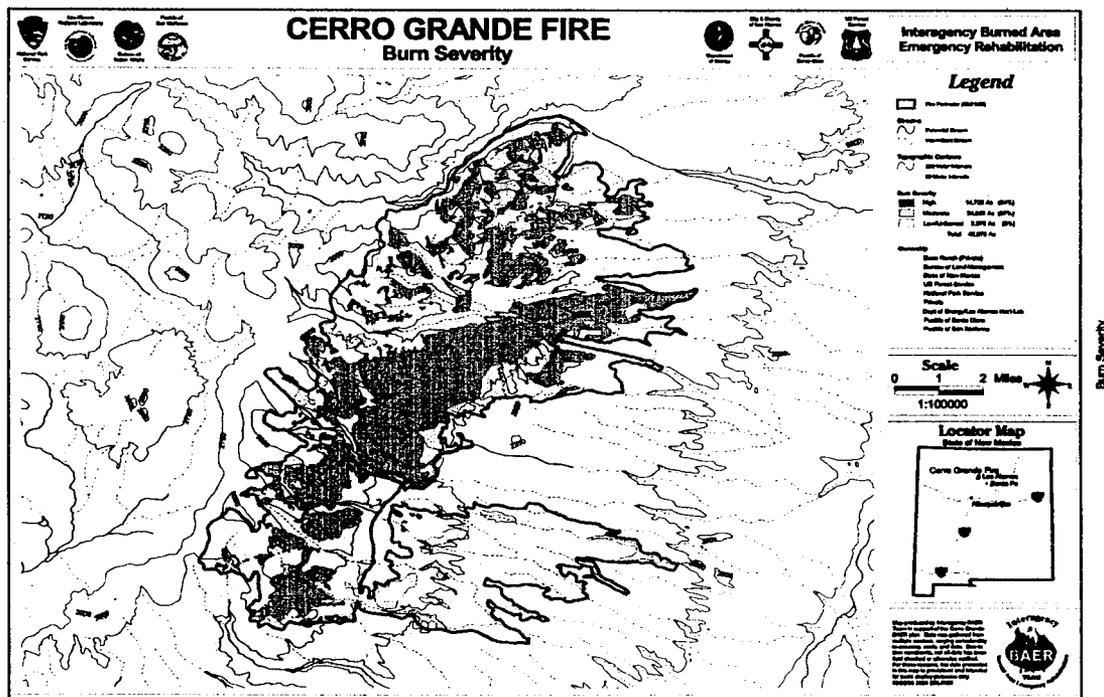


Figure 2. The Cerro Grande fire burn severity map.

Avian Monitoring Network

To investigate health and condition of birds at areas of concern at concern, including Mortandad Canyon, an avian monitoring network of nest boxes was initiated at LANL. During the winter of 1997, 438 nest boxes were placed on LANL in total of 18 both potentially contaminated and reference areas (Fig. 2). Nest boxes 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.

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 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. Sexual dichromatism differs in the two species, with the WEBL being

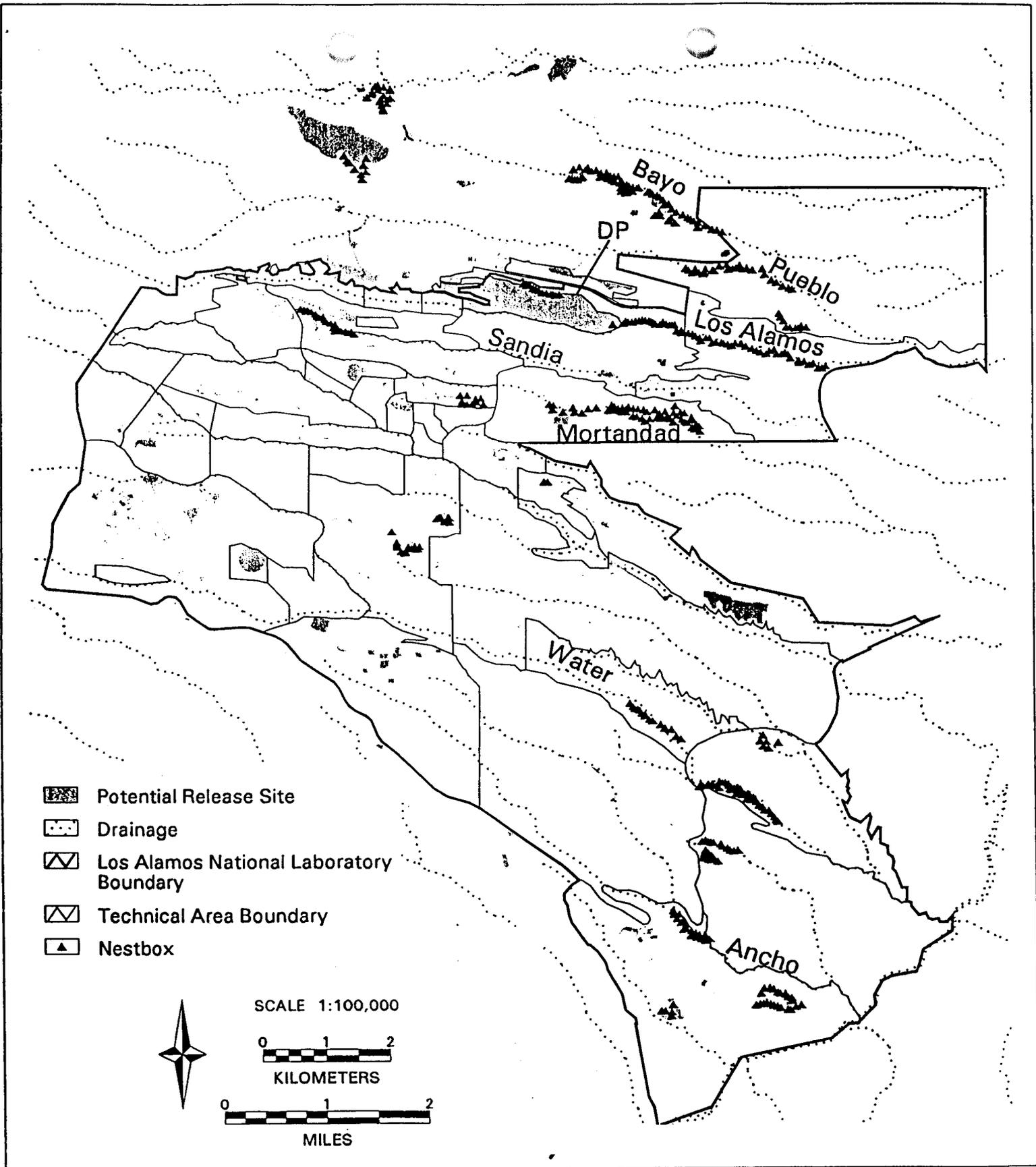


Figure 1. Nestbox monitoring network on Los Alamos National Laboratory. Major locations noted are canyons.

sexually dichromatic and the ATFL having no sexual differences. Although the specific migratory pattern of both species is unknown, WEBLs are more common in the winter months in the study area and it is thought that the ATFLs migrate farther south, even in mild winter years. Both bird species in this study readily utilized nest boxes and are common in northern New Mexico.

The approach described by this study may be used in ecological risk assessments where a hierarchical approach is needed to determine not only which life history traits are most sensitive to contaminants but also which life history traits most affect population dynamics. The sensitivity of a particular species to stochastic fluctuations depends in large part on its life history characteristics, such as age at first reproduction, clutch size, and reproductive life span. Many of these parameters, such as the proportion of females reproducing in a year, can be highly variable and might be influenced by environmental conditions (Fitzgerald, 1994). It is now known that the most sensitive life-history traits do not necessarily reduce fitness; thus, the effect of contaminants on single traits must be evaluated from a life-history perspective to ensure a proper assessment of the potential ecological risk to anthropogenic stress. For example, a toxicant or other external stress factor could dramatically reduce juvenile survival but have negligible effect on fitness, or it could cause minor reductions in juvenile survival but drastically reduce fitness due to the life history of the species.

Methods

The main current objective of the avian nest box-monitoring network is to investigate population level parameters such as survival, nest productivity, and return rates or recruitment into the population. All adults and nestlings western bluebirds are to be banded and return band numbers are recorded. This data will be used in a population viability analysis that can determine the status of the population. The LANL bluebird population will be compared with a western bluebird population in Oregon, California, and Arizona.

The sex of western bluebird nestlings that were 15 days or older was determined by plumage color identification.

Cell-mediated response

Cell-mediated immunity was continued to be measured in nestlings using the dermal phytohemagglutinin (PHA) (Sigma Chemical Company, St. Louis, MO) reaction in the wing web. PHA injected for localized *in vivo* inflammatory response in birds has long been used to measure cell-mediated immunity (Lamont 1984, Stadecker 1977) and has been determined to not impose additional stress or survival cost (Merino et al. 1999, Smits et al. 1999). Birds were inoculated intradermally on day 15 in the wing-web with either 0.05 ml (1.0 mg/ml) PHA in phosphate-buffered saline (PBS) (right side) or 0.05 ml PBS only (left side). A micrometer to the nearest 0.001 mm measured the amount of swelling in the wing-web 24 hours after inoculation. A PHA index was computed as the thickness of the PHA-inoculated wing-web minus the thickness of the

opposite wing-web and standardized by the average wing thickness before inoculation, i.e.,

$$PHA\ Index = \frac{postPHA - postPBS}{\left(\frac{prePHA + prePBS}{2}\right)}$$

Each nestling was handled for less than five minutes in accordance with the Guidelines for the Use of Wild Birds in Research (Gaunt and Oring 1997). The animal care and use committees of both LANL and the University of Missouri-St. Louis approved all protocols.

Data Analysis

The Statistical Analysis System (SAS, Institute, Inc. 1987) was used for all statistical analyses, and assumptions for parametric statistics were examined. Growth and physiological parameters were compared among antigen treatments using repeated measures Analysis of Variance models (ANOVA). Means for each treatment were compared with Duncan's Multiple Range Test. Data not normally distributed or having unequal variances were compared with Kruskal-Wallis nonparametric tests.

RESULTS

Productivity and Survival

In 2001, Mortandad canyon had a higher occupancy rate for active nests than any other location on LANL (Figs. 3 & 4). A total of 48 of 51 boxes were burned in the Cerro Grande fire in Mortandad canyon in 2000. While Mortandad canyon was considered a relatively intense burn for LANL land, it left the majority

of the large Ponderosa pines in the canyon and cleared out most of the very developed underbrush. Mortandad canyon also received rehabilitation seeding by plane and much of 2001 growth was from this seeding. The result for 2001 was a very green and diverse plant base that was visually very different from unburned canyons. Table 1 contains total productivity data for Mortandad canyon from 1997-2001.

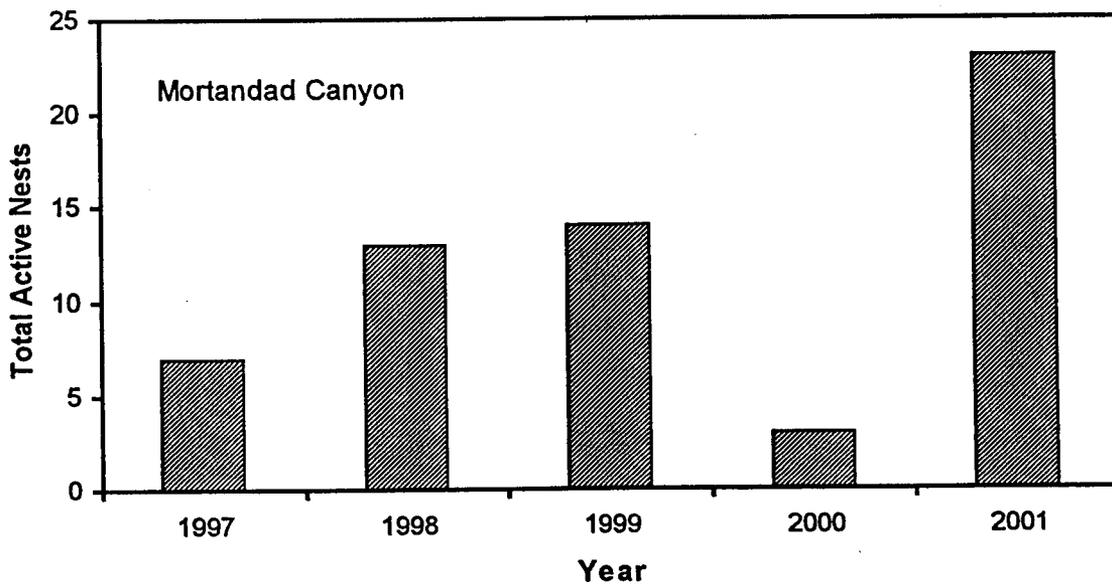


Figure 3. Total active nests for Mortandad Canyon from 1997-2001 for all avian species.

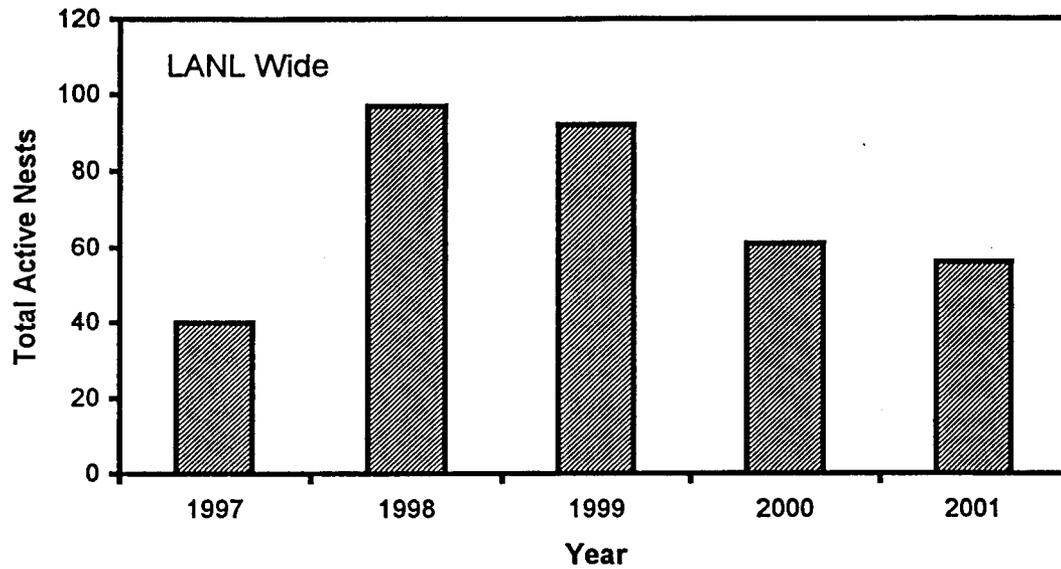


Figure 4. Total active nests for 1997-2001 for the entire laboratory nest box network.

Table 1. Nest box history and occupancy rates for Mortandad canyon.

	1997	1998	1999	2000	2001
Total # Boxes	54	54	54	3	60
				(51 burned)	
Number Occupied	7	13	14	3	23
% Occupied	12.96	24.07	25.93	100	38.33
Species	WEBL ATFL MOBL HOWR VGSW ROWR	WEBL ATFL MOBL HOWR MOCH JUTI	WEBL ATFL HOWR MOCH	WEBL ATFL	WEBL ATFL MOBL HOWR VGSW

The number of active boxes increased from 1997 to 1998 and 1999 for the western bluebird but not for the ash-throated flycatcher, then significantly declined in 2000 and 2001 following the Cerro Grande Fire (Fig. 4). Predators of nestlings included bull snakes (*Pituophis melanoleucus*) piñon mice (*Peromyscus truei*), Colorado chipmunk (*Eutamias quadrivittatus*), and raccoons (*Procyon lotor*) (Fair, personal obs.). Predation for both species increased in 1999 and then decreased slightly in 2001.

Total Active Nests = 83

Species #	WEBL	ATFL	VGSW	HOWR	MOBL	MOCH	HOFI
	31	24	10	10	5	2	0

Table 2. Active nests with eggs by canyon.

Location	WEBL	ATFL	VGSW	HOWR	MOBL	MOCH	HOFI
Mortendad	6	5	4	4	5	-	-
Pueblo	3	3	3	2	-	-	-
LA	1	6	-	-	-	-	1
Bayo	3	1	1	-	-	-	-
DP	3	1	-	-	-	1	-
Sandia	2	1	-	2	-	-	-
Canada del Buey	2	2	1	-	-	-	-
DX	4	-	-	-	-	-	-
Cemetery	2	-	-	2	-	-	-
Golf Course	1	-	1	-	-	-	-
Ancho Mesa	1	1	-	-	-	-	-
Gate 9	1	1	-	-	-	-	-
Water	1	-	-	-	-	-	-
TA-51	1	-	-	-	-	-	-
Portrillo Fence	-	1	-	-	-	-	-
Lower Sandia	-	1	-	-	-	-	-
TA-33	-	-	-	-	-	-	-
TA-35	-	-	-	-	-	-	-
Ancho Canyon	-	-	-	-	-	-	-
A/W Mesa	-	-	-	-	-	-	-

Western bluebird, ash-throated flycatcher, violet green swallow, house wren, mountain bluebird, mountain chickadee, and house finch.

Table 3. Mortality types per species.

	WEBL	ATFL	MOCH	MOBL	HOWR	VGSW
Collected eggs	20	21	3	2	1	1
Dead nestlings	19	7	0	0	0	0

Table 4. Total birds banded (N = 156).

Species	WEBL	ATFL	MOBL	MOCH	HOWR
#	80	45	16	8	7

Cell-mediated response

The cell-mediated immune response of birds for all LANL locations were not significantly different ($F_{127,9} = 1.57, p = 0.131$). The response to PHA for the birds in Mortandad was not different than any other location (Duncan's Multiple Range Test). The only location that had significantly lower cell mediated immune response was DP canyon with an average PHA index of 0.69. Mortandad canyon had a mean PHA index of 1.20.

DISCUSSION

Survival was related to location or breeding sites of the birds. Survival did not vary with year, species, Julian hatch date, brood size, or clutch size. However, it did vary between locations and was lower for flycatchers in areas closer than 300 meters to a PRS. Survival was not, however, significantly correlated with the distance to a PRS. Although survival did not differ between species, flycatchers had less phenotypic variation in survival (11.5%) across all

locations and years compared to the bluebird (20.5%) and flycatchers had lower survival in areas associated with contamination. Although survival varied with respect to contamination sites, none of the parameters measured with respect to growth or immune response varied in parallel. So, there may have been effects, but the mechanisms are not clear. There were several causes of mortality in this study, from snake predation to cold exposure. Specific predators such as snakes may also vary with contamination or habitat. Mortandad canyon did not have a significantly higher amount of mortality.

The WEBL tarsus length did vary among locations and was longer at locations farther away from PRSs and was shortest in Mortandad canyon than all other locations. Tarsus length may be a better predictor of environmental stress and it may be that most tarsus growth occurs early in development and most wing length growth somewhat later. Mock et al. (1991) predicted from its more rapid development rate and higher daily energy requirements (22% higher per day) that the ash-throated flycatcher would be more susceptible to nest failure than the western bluebird. Although the adult mass of both species is similar (range 25-30 g), and nestlings fledge at a similar body mass (27-28 g), the age of fledging differs by 4-5 days (bluebirds in this study 17-22 days; flycatcher 14-16). Ricklefs (1973) proposed that biochemical and molecular constraints might limit the extent which tissues can both differentiate functionally, proliferate, and grow. The flycatcher may become functionally mature at an earlier age to achieve sustained flight soon after its earlier fledging age, even though its growth curve resembles that of the western bluebird. T-cell-mediated response of the

flycatcher was much stronger than the bluebird, suggesting that T-cell development and maturation may parallel that of other tissues and the development of the high field metabolic rate reported by Mock et al. (1991).

Young chicks may be sensitive to disease agents because of the relative immaturity of their immune systems. Pitcovski et al. (1987) point out that although the ability to mount a humoral response to some antigen is present during first week posthatch, the rate of maturation differs among antigens (Matsuda and Bito 1973). Even after some acquired immunity has developed, antibody titers are stronger for older birds (Peleg et al. 1985). Seto and Henderson (1967) showed that antibody responses of chickens to erythrocytes was stronger and earlier for mouse erythrocytes than sheep erythrocytes and did not reach substantial levels until four weeks of age. Due to sensitivity of the agglutination test that we used, the flycatchers and bluebirds had detectable antibodies even though the levels were extremely low.

The ATFL had a stronger response to PHA, suggesting a more rapid development of T-cells than the WEBL. However, the B-cell specific antigens (that were T-cell independent) elicited a similar response between the species. The ontogeny of B-cell development parallels serum immunoglobulin levels, which for the first week in life consist of IgG from maternal contribution (Apanius 1998). Thus, it appears that the T-cells that play a vital role in both humoral and cell-mediated responses are more abundant at fledging age in the ATFL than the WEBL. The impact of stress on the cell-mediated response can be severe, with a reduction in both delayed-type hypersensitivity and cytotoxicity (Kuby 1997).

Defects in the humoral system primarily influences the immune response to infectious encapsulated bacteria and defects in the cell-mediated system are associated with increased susceptibility to viral, protozoan, and fungal infections.

The cell-mediated effects were dynamic in that the same locations in general showed similar patterns for each year. Each year the birds at the cemetery had relatively high PHA response and the longest wing lengths. In contrast, the birds breeding in a site called DP canyon had some of the lowest PHA responses as well as the shortest wing lengths for both years that had breeding birds. Although PHA response represents only one aspect of the immune system, it might not only be critical to maintain a high cell-mediated response owing to its interconnectedness with the humoral response, but it proves to be one of the quickest, most feasible, and least variable method to assess at least T-cells.

Mortandad Canyon

Risk assessment will continue to be somewhat retrospective. While this limits strong inference, confirming treatment—control experiments, can augment assessments. In either case, careful measures of uncertainty are critical in objective risk assessment and management. Long-term life-history studies can be intensive and potentially expensive and, in some cases, technologically impossible. Proactive studies to determine the sensitivities of various life-history traits to contaminants could save millions of dollars in the long run and potentially

Monitoring networks across a landscape of various remediation concerns can offer large impact on decisions as specific questions can then be asked

regarding specific areas. In this project, specific areas of concern such as Mortandad canyon can now be monitored and hypotheses tested for environmental impacts of stressors of concerns endemic to those locations. Also, once the broad stroke view of all of the areas is completed, as in this study, areas of concern can be pinpointed. Then a more focused and detailed study can be designed for that area using more specified contaminant data that can better determine the environmental causes of the perceived decrease in the trait or immunocompetence. Birds breeding in Mortandad canyon have a slightly higher probability of survival than birds breeding in other locations of LANL and this may be due to the habitat of this open canyon with a diversity of edge environments. This also may be evidenced in the high occupancy rates of boxes in this after the Cerro Grande fire where birds are opportunistically selecting this canyon for breeding. It is clear from this research, however, that Mortandad canyon is an important breeding area for cavity nesting birds on the Pajarito Plateau.

The Future

In 2002, this project will be gear specifically toward answering compliance-based issues and question at Los Alamos National Laboratory. It will be expanded to include the area around MDA-P in TA-16 that is of concern for contaminants, runoff, and was a material disposal area just remediated over the last five years. This project will also be a part of a larger population viability analysis of western bluebirds in North America. The data from four other long-term western bluebird projects will be used together to develop a working model

of bluebird population dynamics in four main regions in North America. This will undoubtedly give considerable information on how the population of bluebirds at LANL relatively compare in health and condition and give valuable information on the effects of Cerro Grande Fire.

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Glossary

ATFL	Ash-throated Flycatcher
ELISA	Enzyme linked immunoassay
HOWR	House Wren
JUTI	Juniper Titmouse
MOCH	Mountain Chickadee
NDV	New Castle Disease
PRS	Potential Release Site
ROWR	Rock Wren
SRBC	Sheep Red Blood Cells
VGSW	Violet Green Swallow
WEBL	Western Bluebird

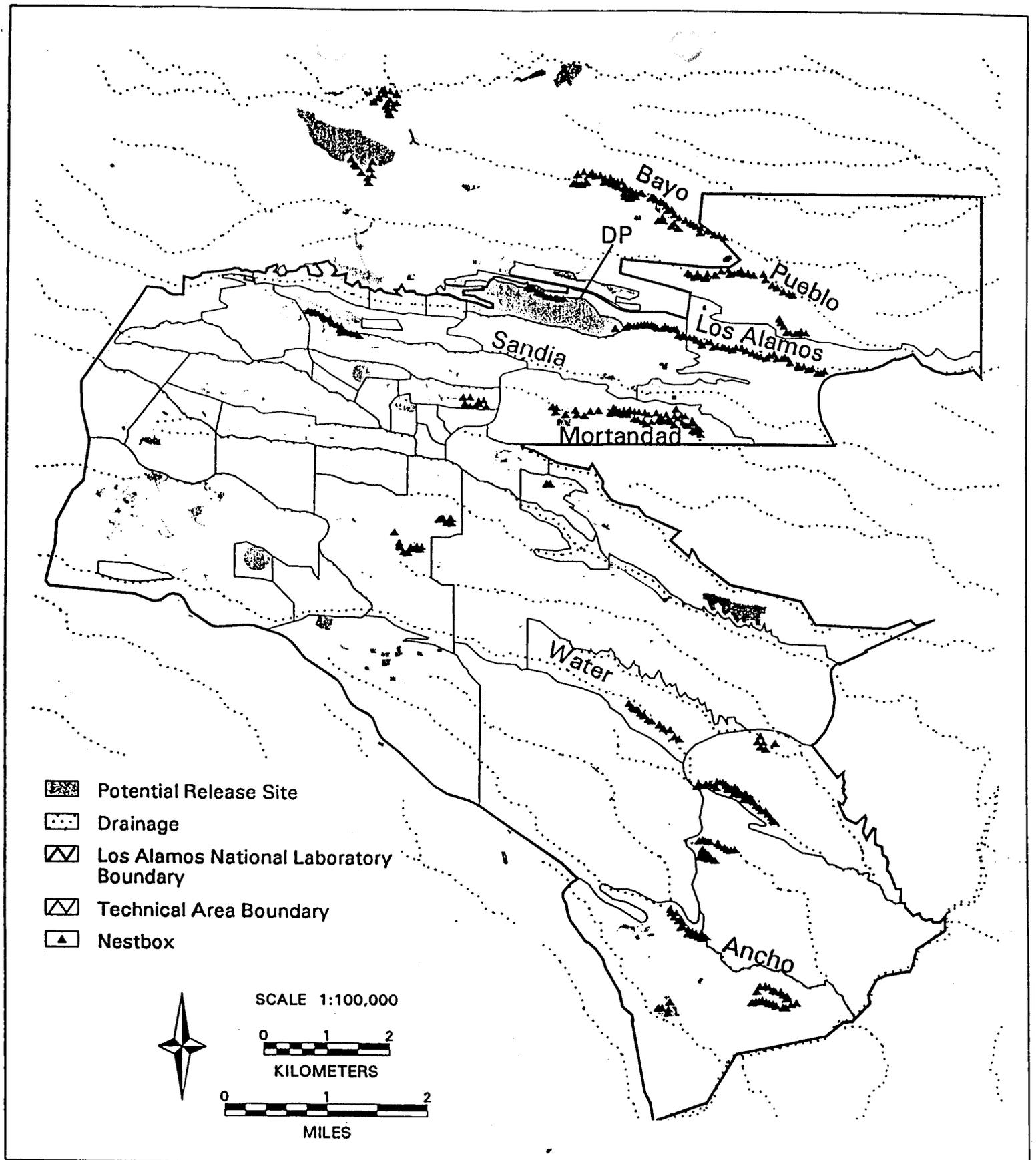


Figure 2. Nestbox monitoring network on Los Alamos National Laboratory. Major locations noted are canyons.