

University of Nebraska - Lincoln

DigitalCommons@University of Nebraska - Lincoln

US Fish & Wildlife Publications

US Fish & Wildlife Service

2005

Secondary Lead Poisoning in Golden Eagle and Ferruginous Hawk Chicks consuming Shot Black-tailed Prairie Dogs, Thunder Basin National Grassland, Wyoming

Robert M. Stephens

Wyoming Cooperative Fish and Wildlife Research Unit

Aran S. Johnson

Wyoming Cooperative Fish and Wildlife Research Unit

Regan Plumb

Wyoming Cooperative Fish and Wildlife Research Unit

Kimberly K. Dickerson

U.S. Fish and Wildlife Service

Mark C. Mckinstry

Wyoming Cooperative Fish and Wildlife Research Unit

See next page for additional authors

Follow this and additional works at: <https://digitalcommons.unl.edu/usfwspubs>



Part of the [Aquaculture and Fisheries Commons](#)

Stephens, Robert M.; Johnson, Aran S.; Plumb, Regan; Dickerson, Kimberly K.; Mckinstry, Mark C.; and Anderson, Stanley H., "Secondary Lead Poisoning in Golden Eagle and Ferruginous Hawk Chicks consuming Shot Black-tailed Prairie Dogs, Thunder Basin National Grassland, Wyoming" (2005). *US Fish & Wildlife Publications*. 230.

<https://digitalcommons.unl.edu/usfwspubs/230>

This Article is brought to you for free and open access by the US Fish & Wildlife Service at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in US Fish & Wildlife Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Authors

Robert M. Stephens, Aran S. Johnson, Regan Plumb, Kimberly K. Dickerson, Mark C. Mckinstry, and Stanley H. Anderson



Contaminants Report Number: R6/720/05

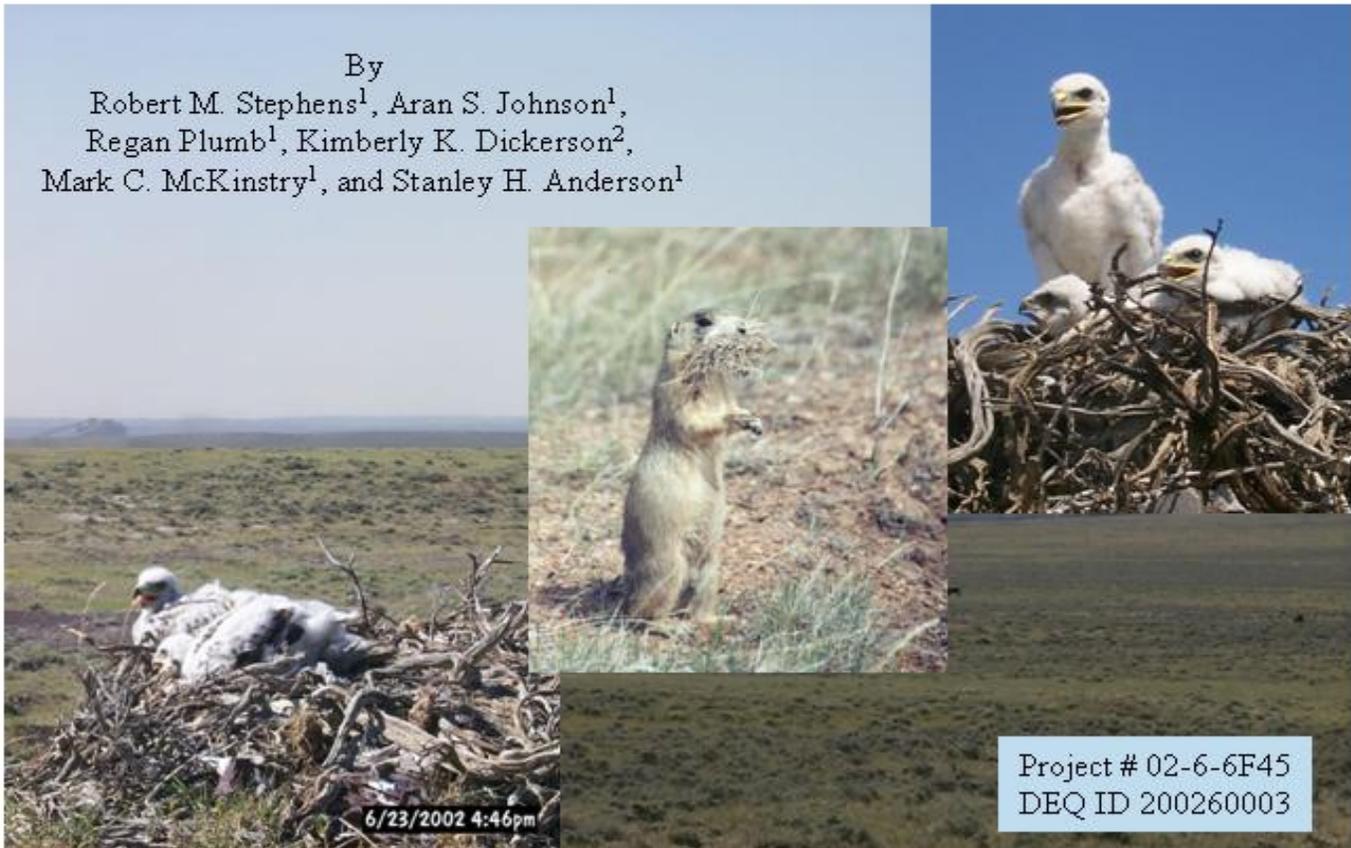
U.S. Fish and Wildlife Service
Region 6
Environmental Contaminants Program



**Secondary Lead Poisoning in Golden Eagle and Ferruginous
Hawk Chicks Consuming Shot Black-tailed Prairie Dogs,
Thunder Basin National Grassland, Wyoming**

By

Robert M. Stephens¹, Aran S. Johnson¹,
Regan Plumb¹, Kimberly K. Dickerson²,
Mark C. McKinstry¹, and Stanley H. Anderson¹



Project # 02-6-6F45
DEQ ID 200260003

¹Wyoming Cooperative Fish
and Wildlife Research Unit
P.O. Box 3166
Laramie, Wyoming 82071

²U.S. Fish and Wildlife Service
4000 Airport Parkway
Cheyenne, Wyoming 82001

December 2005

Abstract

Recreational shooting of black-tailed prairie dogs (*Cynomys ludovicianus*) is a common activity at Thunder Basin National Grassland (TBNG), Wyoming. The prairie dog carcasses left in the area are scavenged by coyotes (*Canis latrans*), raptors, and other animals. These scavengers are susceptible to lead (Pb) poisoning if they consume Pb bullet fragments or Pb shot when scavenging the shooter-killed prairie dogs. In 2000, a local rehabilitator noted an increase of Pb poisoning cases in raptors (L.Layton, pers. comm. 3/30/01) from the area. We collected several shooter-killed prairie dog carcasses from TBNG for determining if Pb fragments remained embedded in the tissue that potentially would be consumed by raptors. Radiographs showed fragments consistent with Pb to be present. In 2002, we conducted a more in-depth study to determine if Pb poisoning was occurring in raptors at TBNG by documenting the number of raptors on prairie dogs at colonies where shooting occurred, assaying bullet fragments in shot prairie dogs to determine Pb content, and analyzing blood and feather samples of ferruginous hawk (*Buteo regalis*) and golden eagle (*Aquila chrysaetos*) nestlings and feathers from burrowing owls (*Athene cunicularia*) for clinical signs of Pb poisoning.

We observed raptors foraging at prairie dog colonies and collected data on the number of shooters shooting at prairie dog colonies. To determine if raptors preferred foraging on shot prairie dogs, we compared raptor use at prairie dog colonies where shooting occurred to raptor use at prairie dog colonies where shooting did not occur. Shooter intensity did not predict raptor use. We also collected prairie dog carcasses and examined them for Pb shot fragments. We detected metal fragments in four of ten prairie dog carcasses. The total weight of the fragments found in each carcass ranged from 10 – 146 mg. Copper was the primary metal detected in 3 of 4 carcasses; but, significant amounts of Pb (20 mg, 28 mg, and 124 mg) were found in the three carcasses. Blood Pb concentrations in ferruginous hawk nestlings were below sub-clinical levels at TBNG and the control site near Rawlins, Wyoming. Analysis of red blood cell delta-aminolevulinic acid dehydratase activity, hemoglobin levels, and protoporphyrin levels also did not indicate Pb poisoning in ferruginous hawk nestlings. Additionally, blood and feather samples from golden eagle nestlings and feather samples from burrowing owls (juveniles and adults) at TBNG did not indicate Pb poisoning. Although ferruginous hawks and golden eagles (and possibly burrowing owls) scavenge on the carcasses of shot prairie dogs and some carcasses contained Pb-bullet fragments, we did not detect Pb poisoning in any of the birds. Lead poisoning may become important if the availability of alternate food sources decreases or shooter intensity increases.

TABLE OF CONTENTS

INTRODUCTION	1
METHODS	3
Study sites.....	3
Raptor use of prairie dog colonies.....	3
Bullet fragments in prairie dog carcasses.....	5
Blood and feather sampling.....	5
Statistical analysis	7
RESULTS	7
Raptor use of prairie dog colonies.....	7
Bullet fragments in prairie dog carcasses.....	8
Blood and feather analysis	8
DISCUSSION.....	13
Raptor use of prairie dog colonies.....	13
Bullet fragments in prairie dog carcasses.....	13
Blood and feather analysis	14
Acknowledgments.....	16
REFERENCES	16

TABLES

Table 1. Number of ferruginous hawks (FeHa) and golden eagles (GoEa) using black-tailed prairie dog colonies in Thunder Basin National Grasslands, Wyoming, during June and July, 2002.....7

Table 2. Assay results for lead (Pb), copper (Cu), and zinc (Zn) of bullet fragments recovered from black-tailed prairie dog carcasses collected from Thunder Basin National Grasslands, Wyoming, July, 2002.....8

FIGURES

Figure 1. Location of Thunder Basin National Grassland and the reference area in Wyoming.....4

Figure 2. Range and median blood lead (Pb) concentrations (µg/g wet weight (ww)) in golden eagles (GOEA) and ferruginous hawks (FEHA) from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002. The lowest observed adverse effect level (LOAEL) is 0.2 ug/g ww.....9

Figure 3. Range and median of blood parameters in ferruginous hawks (FEHA) from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002.....10

Figure 4. Range and median lead (Pb) concentrations in ferruginous hawk (FEHA) and golden eagle (GOEA) nestling feathers from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002. The one down feather collected from the golden eagle nestling with a Pb concentration of 1, 070 ug Pb/g is not shown graphically.....11

Figure 5. Range and median lead (Pb) concentrations in feathers from adult and juvenile burrowing owls from Thunder Basin National Grassland (TBNG), Wyoming, July 2002.....12

APPENDICES

Appendix 1. Activity observations of raptor species using black-tailed prairie dog colonies in Thunder Basin National Grasslands, Wyoming, during June and July, 2002.....20

Appendix 2a. Ammunition type and assay results for lead (Pb), copper (Cu), and zinc (Zn) of bullet fragments recovered from black-tailed prairie dog carcasses collected from Thunder Basin National Grasslands, Wyoming, July, 2002.....21

Appendix 2b. Radiograph examples of prairie dog carcasses collected in July 2000.....23

Appendix 2c. Letter from veterinarian, Dr. Todd Cornish, of the Wyoming State Veterinary Laboratory in Laramie, Wyoming, regarding fragments in shot prairie dog carcasses from Thunder Basin National Grassland, July 2000.....26

Appendix 3a. Nest identification and location, number of nestlings, blood sample identification, and fledgling data for golden eagle (GoEa) and ferruginous hawk (FeHa) nestlings at Thunder Basin National Grassland, Wyoming.....27

Appendix 3b. Band information, fate of bird, date of fledging, age of bird when blood sample was collected from golden eagle (GoEa) and ferruginous hawk (FeHa) nestlings at Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming.....28

Appendix 3c. Blood lead (Pb), hematocrit, and hemoglobin results from golden eagle (GoEa) and ferruginous hawk (FeHa) nestlings at Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming.....31

Appendix 3d. δ -aminolevulinic acid dehydratase (ALAD) and protoporphyrin readings of blood samples from golden eagle (GoEa) and ferruginous hawk (FeHa) nestlings at Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming.....34

Appendix 3e. Lead (Pb) concentrations in feathers from golden eagle (GoEa) and ferruginous hawk (FeHa) nestlings at Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming.....37

Appendix 3f. Band information, age of juvenile bird, and lead (Pb) concentrations in feathers from burrowing owls at Thunder Basin National Grassland, Wyoming.....39

Appendix 3g. Band information, sex of adult bird, and lead (Pb) concentrations in feathers from burrowing owls at Thunder Basin National Grassland, Wyoming.....40

INTRODUCTION

The Thunder Basin National Grassland (TBNG), managed by the U.S. Forest Service (USFS), is located 40 miles north of Douglas, Wyoming. TBNG provides habitat for white-tailed deer (*Odocoileus virginianus*), mule deer (*Odocoileus hemionus*), pronghorn antelope (*Antilocapra americana*), and numerous small mammals. There are 6,177 ha of black-tailed prairie dog (*Cynomys ludovicianus*) towns, of which 1,388 are managed as retention areas (where no control activities occur) at TBNG. The remaining 4,788 ha can have prairie dog control activities, which include shooting or poisoning using zinc phosphide (USFS and BLM 1992).

The prairie dog colonies serve as a locally important food source for raptors. Golden eagles (*Aquila chrysaetos*) and ferruginous hawks (*Buteo regalis*) nest on TBNG. Approximately 100 bald eagles (*Haliaeetus leucocephalus*) use the area for winter roosting, and feed on prairie dogs and winter-kill animals. Golden eagles also feed on winter-kill animals; and in the summer, golden eagles and ferruginous hawks feed on shooter-killed prairie dogs almost exclusively (Tim Byer, USFS, pers. comm. 5/25/00). Annual use by prairie dog-shooters of TBNG ranges from 8,000 to 8,500 shooter-use-days. Prairie dog shooters do not collect the prairie dog carcasses resulting in an easy food resource for the raptors.

As a result of scavenging on shooter-killed prairie dogs, raptors may be exposed to chronic lead (Pb) concentrations by ingesting Pb pellets or Pb-core bullet fragments that are embedded in the tissues of the prairie dog carcasses. Although raptors often regurgitate bones, feathers or fur, and other foreign objects, Redig et al. (1980) found that Pb is not necessarily incorporated into castings and can be retained in the gastrointestinal tract resulting in Pb poisoning. In 2000, several golden eagles with suspected Pb poisoning were brought to a rehabilitator in nearby Casper, Wyoming (L. Layton, raptor rehabilitator, pers. comm. 3/30/01). These cases raised questions about the impacts of recreational shooting on birds of prey.

There is sufficient documentation of raptors being poisoned from ingesting Pb shot in waterfowl tissues (Benson et al. 1974; Jacobsen et al. 1977); but, documentation of Pb poisoning in raptors that consume shooter-killed small mammals is more limited (Locke et al. 1969; Pattee et al. 1981). Even so, it is possible for raptors to suffer from Pb poisoning after consuming small mammal carcasses containing Pb shot or fragments. For example, an Andean condor (*Vultur gryphus*) was poisoned after eating shooter-killed rabbits and ground squirrels (Locke et al. 1969). In the spring of 2000, three reintroduced California condors (*Gymnogyps californianus*) were found dead in Arizona as a result of Pb poisoning and two other were being treated (USFWS 2000). One of the condors had 17 Pb shotgun pellets in its digestive system. Another study conducted on wintering bald eagles in a Utah desert, found that eagles consuming shooter-killed jackrabbits ingested Pb shot and bullet fragments (Platt 1976). Furthermore, a report by Craig et al. (1990) reported that 10 of 17 golden eagles and seven of eight bald eagles that they found in Idaho were exposed to Pb. One of the golden eagles contained a fragment from a copper-

jacketed Pb bullet. The authors stated their sample was biased because they sampled only sick and dead birds, but they believe that Pb poisoning may be a more serious problem in golden eagles than originally identified. Finally, the U.S. Fish and Wildlife Service (1986) reported that shooter-killed jackrabbits consumed by bald eagles from the Great Plains, the High Plains of Texas, and the Black Hills of South Dakota may constitute a major source of Pb to these raptors.

Birds suffering from Pb poisoning are often weak and may ultimately die from another cause such as predation, disease, or starvation (Jacobsen et al. 1977). In a study by Pattee et al. (1981), five bald eagles were dosed with #4 Pb shot. Four of the five birds died and the stomach of each bird contained at least one Pb shot at the time of death. The fifth eagle survived but went blind and was eventually sacrificed. The individual response of the eagles to Pb shot was variable and probably the result of the interaction of various factors such as the number of shot retained, amount of Pb eroded, and individual susceptibility (e.g., health, age).

Lead is typically not transferred from the adults to the young through egg-laying (Pattee 1984). However, young can ingest Pb pellets or fragments embedded in the tissue of food brought to the nest by the parents. Hoffman et al. (1985a) reports that nestlings of kestrels and other altricial birds, such as eagles, exposed to Pb are expected to be impacted to a greater degree (e.g. impaired survival and growth) than precocial species, such as mallards (*Anas platyrhynchos*), because of the delay in development and growth.

Raptors can be evaluated for Pb poisoning using non-lethal means by collecting blood samples. General guidelines, based on associated physiological effects, exist to categorize Pb concentrations in whole blood samples from Falconiformes (Franson 1996). Additionally, the activity of δ -aminolevulinic acid dehydratase (ALAD) in erythrocytes, one of several enzymes necessary for heme synthesis, can be measured to determine exposure to Pb (Leonzio and Fossi 1994). Lead inhibits the activity of this enzyme (Pain 1996) and blood Pb concentrations are directly correlated with ALAD inhibition (Mayer et al. 1992). The use of ALAD in raptors exposed to Pb is documented (Franson et al. 1983; Hoffman et al. 1985a; and Hoffman et al. 1985b) and has become a standard technique in diagnosing Pb poisoning in birds (Leonzio and Fossi 1994, Pain 1995, Henny et al. 2000). Over-exposure to Pb is also known to cause reduced total-blood hemoglobin concentration, elevated levels of protoporphyrin, and decreased packed cell volume (PCV) (Franson 1986; Pain 1995).

To determine if eagles and ferruginous hawks at TBNG are being impacted by ingesting Pb shot from prairie dog carcasses, we established the following objectives: 1) Quantify raptor activities (including feeding, resting, landing) on hunted and non-hunted prairie dog towns on TBNG; 2) Radiograph a random sample of shooter-killed prairie dogs for the presence of Pb pellets/bullet fragments and assay recovered fragments for Pb verification; and, 3) Obtain blood and feathers from nestling raptors feeding on shooter-killed prairie dogs for Pb concentrations and comparison with nestlings not feeding on shooter-killed dietary items.

METHODS

Study Sites

TBNG was selected as the treatment site (Figure 1) due to heavy use of the area for recreational prairie dog shooting. It is administered as part of the Medicine Bow National Forest by the USFS and is located in northeast Wyoming within Campbell, Weston, Converse, and Niobrara counties. TBNG covers 231,488 ha of National Forest System land intermingled with Bureau of Land Management (BLM), State, and private lands. The topography is characterized by moderately level plains, rolling hills, and steep escarpments. Precipitation is <30 cm/year and elevation ranges from 1,370 to 1,600 m. The dominant vegetation is Wyoming big sagebrush (*Artemisia tridentata wyomingensis*), needle-and-thread grass (*Stipa comata*), blue grama (*Bouteloua gracilis*), western wheatgrass (*Agropyron smithii*), ponderosa pine (*Pinus ponderosa*), and cottonwood (*Populus* spp.) corridors.

The reference site, located in south central Wyoming near Rawlins (Figure 1), is characterized as a high, cool desert with <30 cm of precipitation/year and an elevation ranging from 1,829 to 2,134 m. Sagebrush communities are the most common type of vegetation. Other assorted community types are interspersed throughout the landscape including sagebrush/mountain shrub, saltbush steppe, greasewood lowlands and badlands.

Raptor Use of Prairie Dog Colonies

We monitored eight prairie dog colonies in TBNG during six observation periods, including colonies where shooting did not occur. Observation periods lasted three hours and we recorded raptor activity at 10-minute intervals and included the amount of time raptors hunted, fed, or rested in the area. Observations were distributed during the day so that two observations were performed between 0700 and 1100 hrs, 1101 and 1400 hrs, and 1401 and 1800 hrs. Observations occurred between June 7 and July 20, 2002 and were randomly distributed. Shooter intensity was estimated from information collected from voluntary surveys at each colony. Shooters were interviewed and the information on gun caliber and bullet types used for shooting was recorded. Simple linear regression was used to determine if raptor use was predicted by shooter hours and colony size. This information was required to document the extensive and almost exclusive use of the shooter-killed prairie dogs as a primary food source for raptors on TBNG.

WYOMING

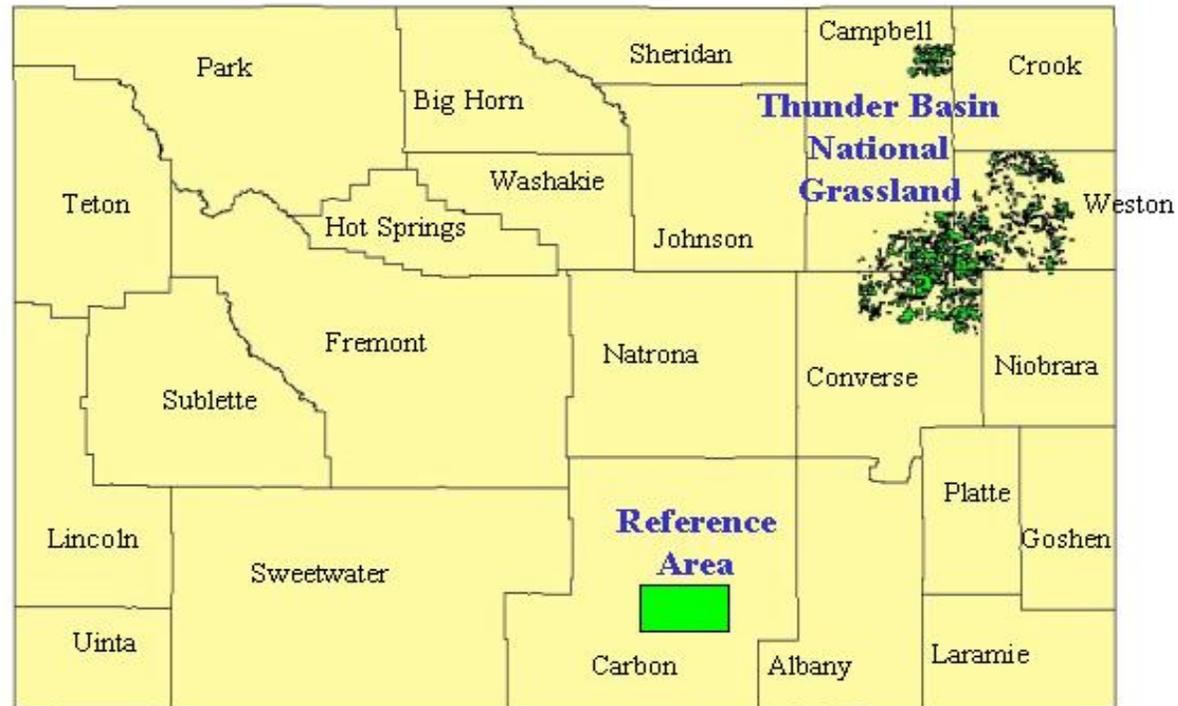


Figure 1. Location of Thunder Basin National Grassland and the reference area in Wyoming.

Bullet Fragments in Prairie Dog Carcasses

As a preliminary investigation to demonstrate that most ammunition used to shoot prairie dogs does not exit the carcasses but rather is retained in fragments, USFWS personnel collected 22 prairie dog carcasses during July 2000 at TBNG. The carcasses were radiographed at the Wyoming State Vet Laboratory (WSVL) to demonstrate that bullet fragments were contained in the tissue. Funding was not available to analyze the content of the fragments from these carcasses. In 2002, ten additional prairie dog carcasses were collected and radiographed by WSVL for the presence of bullet fragments. The metal fragments were removed from the carcasses and assayed by digestion in HNO₃ and H₂O₂, diluted and analyzed on an Elan 6100 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Perkin Elmer, Norwalk, CN) at the WSVL, according to their Standard Operating Procedure (WSVL 3/21/01).

Blood and Feather Sampling

The USFS has monitored nesting success of golden eagles and ferruginous hawks on TBNG each year since 1981 (USFS and BLM 1992). There are currently 30 active golden eagle nests and 25 ferruginous hawk nests at TBNG. We also used information from coal mines to locate active nests at nearby mining sites. Other nests were located using information obtained from Thunder Bird Wildlife Consulting (Wright, WY) and by locating undocumented nest sites. We monitored egg-laying twice weekly from February 2002 until July 2002 when fledgling occurred. Nests were observed through binoculars to reduce disturbance and stress to the birds.

Reference values for Pb concentrations in blood and ALAD levels in raptors is scarce, so samples from ferruginous hawks nesting on platforms on BLM land in south central Wyoming were obtained. The BLM has monitored nest success and productivity of the ferruginous hawk nests for several years. Shooting of prairie dogs does not occur in this area and the raptors' primary diet consists of ground squirrels. We were not able to obtain any golden eagle nestlings for reference birds.

Once nestlings were old enough (approximately 20 d), we collected blood for five hematological analyses, which included blood-Pb concentration, ALAD activity, hemoglobin levels, protoporphyrin levels, and packed cell volume (PCV). PCV readings are required for ALAD analysis. To collect a blood sample, the brachial artery of the nestling was swabbed with alcohol. Two milliliters of blood was obtained using a 25-gauge needle syringe containing heparin-treated beads (USFWS 1996). Immediately after the syringe was removed from the puncture site, we used a heparinized capillary tube to collect blood for measuring PCV. Pressure was applied to the puncture site until bleeding stopped. The nestlings were weighed, and the length of the 8th primary and the width of the footpad were measured to determine age and sex. We collected some chest pin feathers and placed them in resealable bags. Down feathers were collected from nestlings that had not developed pin feathers at the time of sampling. We placed migratory bird identification and Wyoming state bands on their legs and returned the nestlings to their nest.

Samples in capillary tubes were spun by micro-centrifuge for 3 min. The PCV was calculated as the ratio between the hemoglobin of the whole blood and the hemoglobin of packed red blood cells with all plasma removed. For the remaining sample, we divided the 2.0 ml of blood into four sterile heparinized cryogenic vials, each containing a 0.5-ml aliquot of blood. Samples for Pb concentration, hemoglobin, and ALAD were immediately placed into liquid nitrogen and frozen.

Blood Pb analysis, including analysis for percent moisture, was performed at the University of Wyoming Red Buttes Environmental Biology Laboratory (RBEBL; Laramie, WY) on a Varian SpectrAA600 graphite furnace atomic absorption spectrophotometer equipped with Zeeman background correction (Fernandez and Hillgoss 1982). An unused syringe was also analyzed to ensure no external contamination was present.

Hemoglobin analyses were conducted at Antech Diagnostic (Irvine, CA) by the cyanmethemoglobin method and spectrophotometric measurement (Sari et al. 2001). ALAD activity was measured colorimetrically by the National Wildlife Health Center (Madison, WI) with a Beckman DU-65 (Beckman Instruments, Fullerton, CA) spectrophotometer, based on methods described by Burch and Siegel (1971). ALAD activity was determined with duplicate 0.1-ml aliquots of blood; the results reported are the mean of the duplicates. One unit of enzyme activity is defined as an increase in absorbance at 555 nm of 0.100, with a 1.0-cm light path/ml of erythrocytes/hour at 38° C. Protoporphyrin levels were measured with a hematofluorometer at 2 h, 24 h, and 48 h after blood collection according to Franson et al. (1996).

For Pb analysis in feathers, the feathers were washed vigorously in 50% aqueous acetone, followed by three rinses with deionized water to remove loosely adherent external contamination. After washing, feathers were air dried overnight at 60° C and weighed to the nearest 0.1 mg. Weighed samples were digested at 180° C for 10 min. with a combination of 0.5-ml each H₂O₂ and HNO₃ in a microwave digestion system (MDS 2000, CEM Corp, Mathews, NC), cooled, and diluted to 5 ml with deionized water. The diluted samples were analyzed together with appropriate standards, reference samples, and Pb-spiked duplicates by ICP-MS at the WSVL.

Because the opportunity arose, we also analyzed feathers from burrowing owls located at TBNG. Sarah Lantz, with the University of Wyoming, was conducting unrelated research on burrowing owls and collected chest pin feathers from owls that foraged at prairie dog colonies subjected and not subjected to Pb shot at TBNG. Burrowing owls were located with standardized call-broadcast surveys, and captured with two-way traps and baited spring traps.

For data analysis, if Pb concentrations were below the instrument's detection limit (0.001 ppm), the median of the detection limit and zero was used as an individual Pb concentration. Blood Pb concentrations were compared and classified with baseline levels and ranges in *Falconiformes* as established by Franson (1996).

Statistical Analysis

Ninety-five percent confidence intervals and graphs were created using Sigma Plot (version 7.0). Pearson correlation coefficients were calculated using Systat (version 10.2) to determine if correlation existed between blood Pb concentrations and feather Pb concentrations.

RESULTS

Raptor Use of Prairie Dog Colonies

During 144 h of observations, ferruginous hawks (0.36/hr), golden eagles (0.34/hr), turkey vultures (0.27/hr; *Cathartes aura*), red-tailed hawks (0.09/hr; *Buteo jamaicensis*), American kestrels (0.05/hr; *Falco sparverius*), prairie falcons (0.02/hr; *Falco mexanicus*), and Swainson's hawks (0.02/hr; *Buteo swainsoni*) were observed either flying or perched at prairie dog colonies (Table 1; Appendix 1). We did not observe any of the raptors feeding on prairie dog carcasses. Shooter hours ($n = 8$; $r^2 = 0.2\%$; $P = 0.921$) and colony size ($n = 8$; $r^2 = 0.3\%$; $P = 0.894$) were not predictors of raptor use.

Table 1. Number of ferruginous hawks (FeHa) and golden eagles (GoEa) using black-tailed prairie dog colonies in Thunder Basin National Grasslands, Wyoming, during June and July, 2002.

Prairie Dog Colony	Size of Prairie Dog Colony (ha)	Shooter Hours	Number of FeHa and GoEa Observed	Number of All Raptors Observed
Teckla	42	4	36	41
Reservoir Dogs	17	11.5	18	21
Big Steckley	121.3	63	4	28
Little Steckley	15.8	0	5	6
450 East	12.5	17	1	4
BT450	89	72.5	19	31
Mackey	27.4	2	9	17
Rochelle Hills	23.8	2	7	16

Bullet Fragments in Prairie Dog Carcasses

The objective was to collect fifty shooter-killed prairie dogs during June 2002 but only ten prairie dog carcasses could be salvaged as prairie dog populations were low and shooter activity was minimal compared to other years. We found bullet fragments in four of 10 carcasses (Table 2; Appendix 2a). The total weight of the bullet fragments recovered from each carcass was 92.5 ± 60.7 mg (Mean \pm SD; $n = 4$). Significant amounts of Pb were in three carcasses.. Copper was the primary metal ($\geq 78\%$) in three samples and was accompanied by traces of zinc. Funding was not available to analyze the fragments from the 22 carcasses collected during the preliminary investigation in July 2000. However, of the 22 prairie dog carcasses, 19 had fragments that were consistent with Pb (Appendix 2b and 2c).

Table 2. Assay results for lead (Pb), copper (Cu), and zinc (Zn) of bullet fragments recovered from black-tailed prairie dog carcasses collected from Thunder Basin National Grasslands, Wyoming, July, 2002.

Sample	Rifle Caliber	Total Shot Weight (mg)	Pb Content		Cu Content		Zn Content
			% ^a	mg	% ^a	mg	% ^a
1	0.22-250	146	19	28	78	113	<1
2	0.22-250	10	<1	---	106	10	<1
3	0.25-06	85	23	20	79	67	<1
4	0.25-06	129	96	124	<1	---	<1

^a Given the very high concentrations of Pb and Cu, the samples had to be repeatedly diluted in order to estimate the percentages of each metal; thus the numbers do not add up to exactly 100%.

Blood and Feather Analysis

Twenty three ferruginous hawk nestlings (26.5 ± 3.2 days old) (Appendices 3a and 3b) were sampled for blood and feather samples at nine nests in TBNG. We also collected seven blood samples and six feather samples from seven golden eagle nestlings in TBNG. At the control site near Rawlins, blood and feather samples were collected from 23 ferruginous hawks nestlings (39.2 ± 2.8 days old) at nine nests (Appendices 3a and 3b).

Ferruginous hawk ($0.021 - 0.061$ μg Pb/g wet weight (ww) at TBNG; $0.023 - 0.167$ μg Pb/g ww at Rawlins) and golden eagle ($0.03 - 0.074$ μg Pb/g ww at TBNG) blood samples were below sub-clinical levels (Franson 1996) of 0.2 to 1.5 μg Pb/g ww (Figure 2; Appendix 3c). The ALAD activity (215 – 460 Burch & Siegel units), hemoglobin levels (5.5 – 15.9 units), and protoporphyrin levels (5 – 34 units at 48h) in ferruginous hawk nestlings at TBNG also suggested against Pb poisoning (Figure 3; Appendices 3c and 3d) and were not significantly different from the ALAD activity (242 – 339 Burch & Siegel units), hemoglobin levels (9.1 –

15.3 units), and protoporphyrin levels (6 – 17 units at 48h) in ferruginous hawk nestlings at the Rawlins site. However, PCV in ferruginous hawk nestlings was higher at the Rawlins site (35.17 – 37.25) than in TBNG (29.13 – 31.75). The 95% confidence intervals for the other hematological parameters in blood samples from the golden eagles were as follows: ALAD activity = 404.3 – 528.5, protoporphyrin levels at 48 h = -7.2 – 79.0, hemoglobin levels = 7.87 – 11.59, and PCV = 25.46 – 35.1.

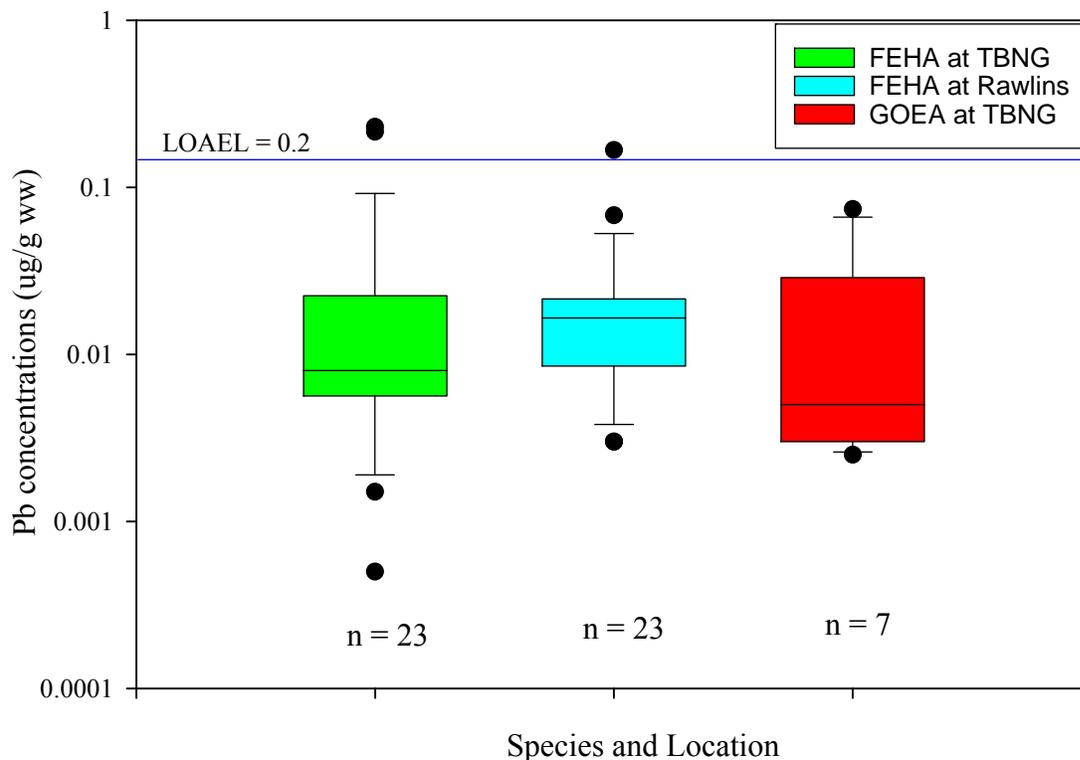


Figure 2. Range and median blood lead (Pb) concentrations ($\mu\text{g/g}$ wet weight (ww)) in golden eagles (GOEA) and ferruginous hawks (FEHA) from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002. The lowest observed adverse effect level (LOAEL) is 0.2 $\mu\text{g/g}$ ww.

Pb concentrations in feathers of ferruginous hawk nestlings from TBNG ranged from 0.08 – 24.72 $\mu\text{g/g}$ for pin feathers and 0.183 – 1.306 $\mu\text{g/g}$ for down feathers. At the reference site, all pin feathers were collected from ferruginous hawk nestlings with Pb concentrations ranging from 0.048 – 2.616 $\mu\text{g/g}$ (Figure 4; Appendix 3e). Pin feathers from golden eagle nestlings on TBNG had Pb concentrations ranging from 0.101 to 1.935 $\mu\text{g/g}$. The one and only down feather sample collected from a golden eagle nestling on TBNG had the highest Pb concentration (1,070 $\mu\text{g/g}$) of all feathers from raptors collected (Figure 4; Appendix 3e).

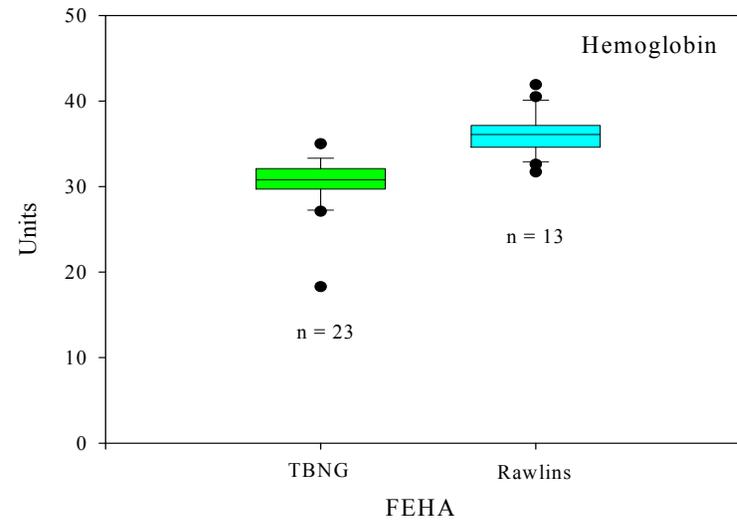
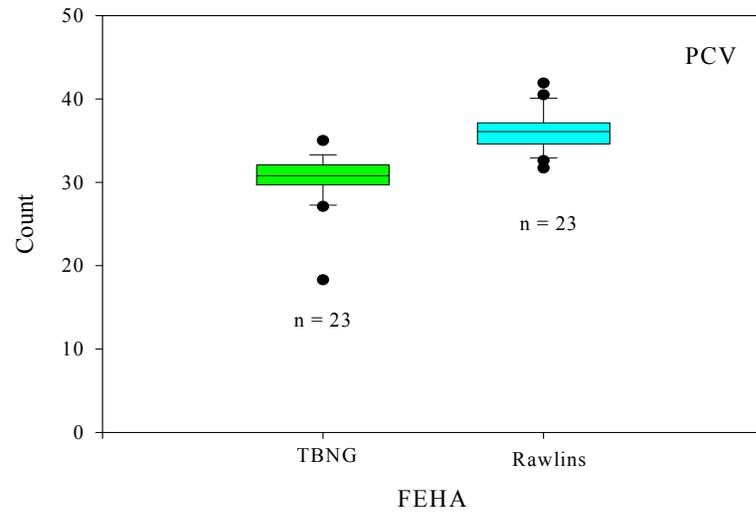
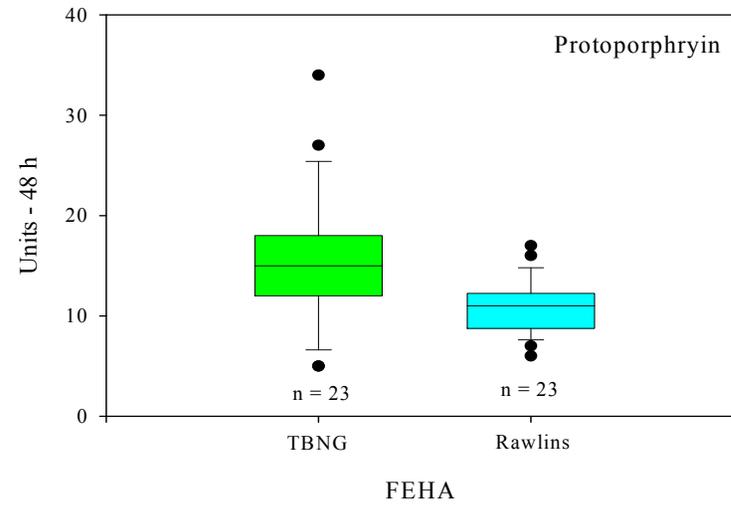
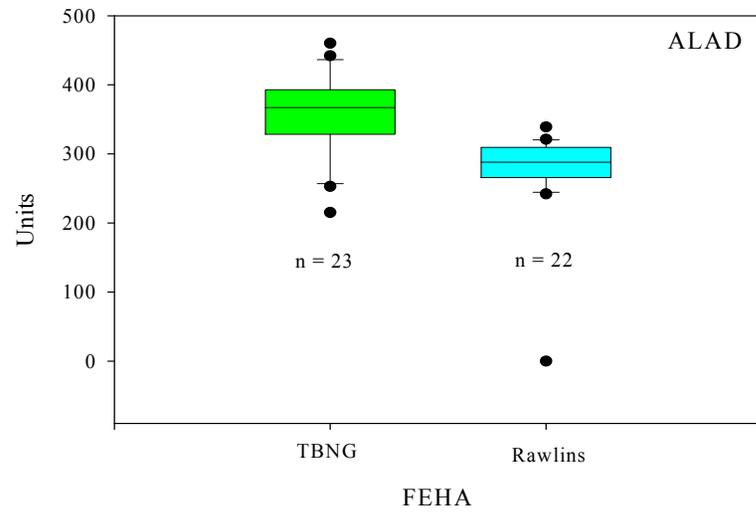


Figure 3. Range and median of blood parameters in ferruginous hawks (FEHA) from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002.

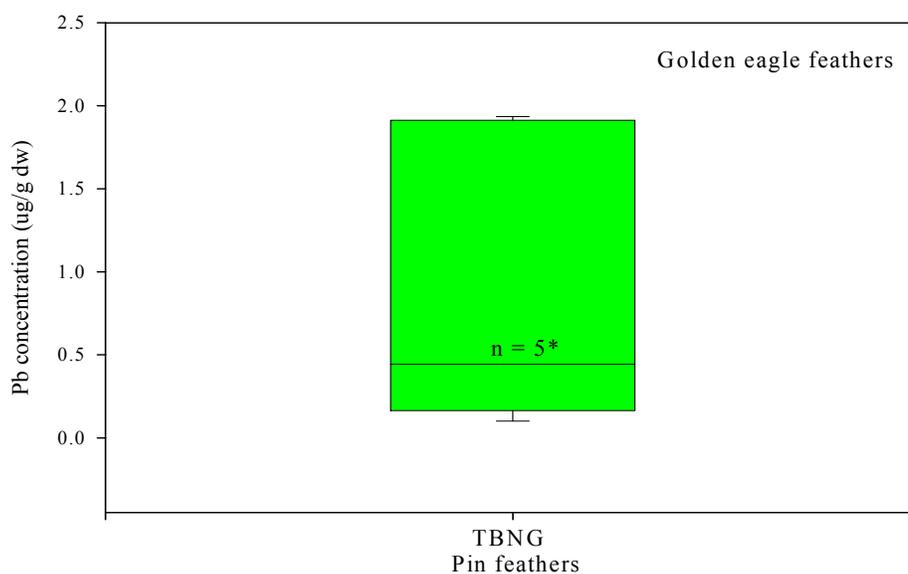
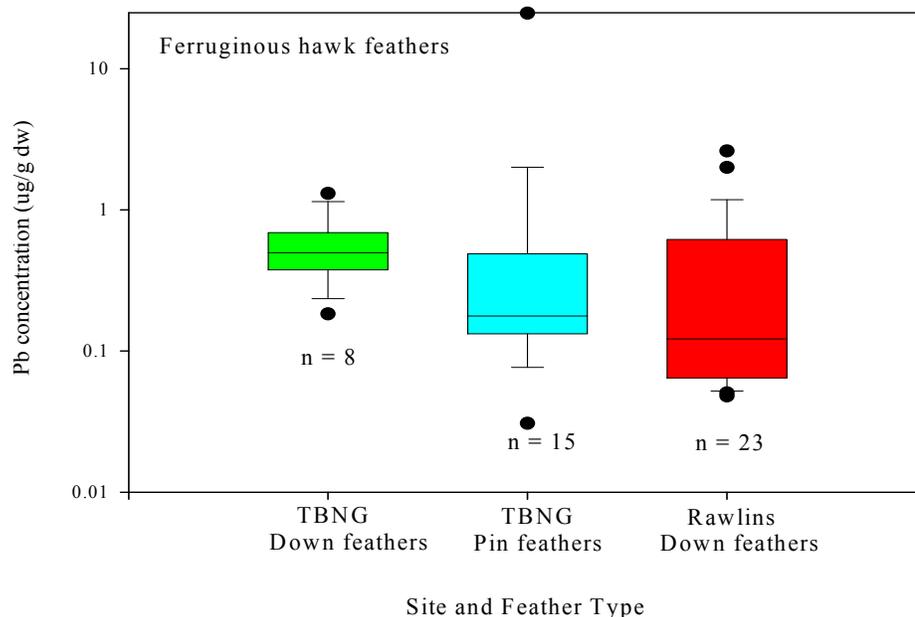


Figure 4. Range and median lead (Pb) concentrations in ferruginous hawk (FEHA) and golden eagle (GOEA) nestling feathers from Thunder Basin National Grassland (TBNG) and the reference site (Rawlins), Wyoming, July 2002. The one down feather collected from the golden eagle nestling with a Pb concentration of 1,070 ug Pb/g is not shown graphically.

Chest pin feathers from 43 burrowing owls, 20 from shot prairie dog colonies [7 juvenile/13 adult] and 23 from unshot prairie dog colonies [12 juvenile/11 adult] in TBNG, were also low in Pb and there was no statistical difference in feathers of owls collected on shot or unshot prairie dog towns (Figure 5; Appendices 3f and 3g).

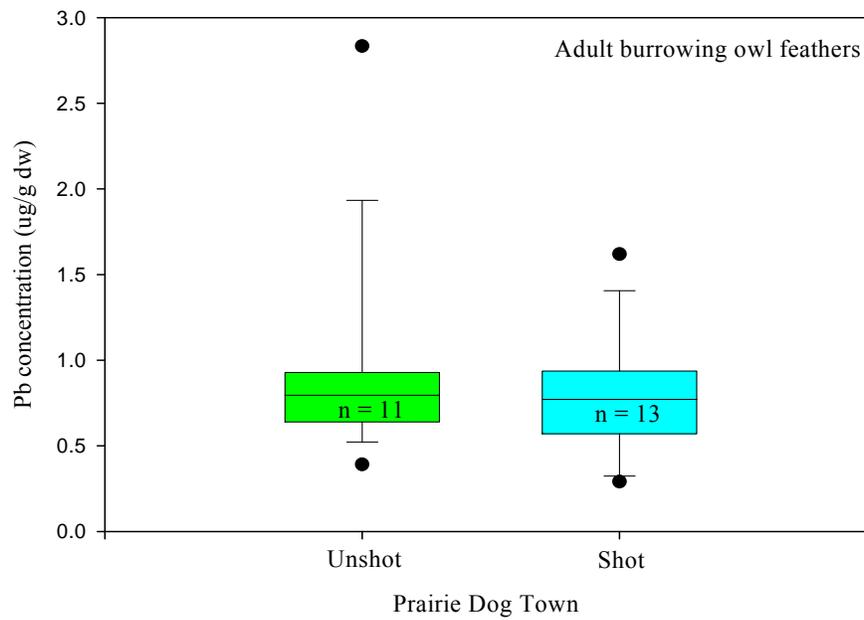
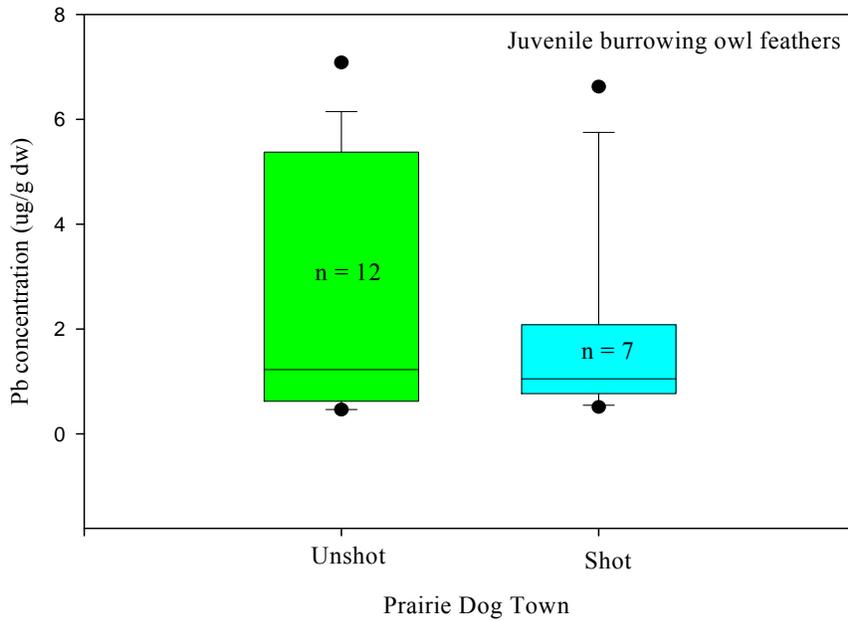


Figure 5. Range and median lead (Pb) concentrations in feathers from adult and juvenile burrowing owls from Thunder Basin National Grassland (TBNG), Wyoming, July 2002.

Chest pin feathers of juvenile burrowing owls ranged from 0.512 to 2.27 ug Pb /g at shot prairie dog towns and from 0.46 – 7.08 ug Pb/g at unshot prairie dog towns. Adults had lower ranges of Pb concentrations in their feathers than juveniles from both shot (0.291 – 1.62 ug Pb/g) and unshot shot prairie dog towns (0.391 – 2.83 ug Pb/g). Differences in Pb concentrations in feathers, however, were not significantly different between adult and juvenile feathers ($p = 0.07$; $p = 0$).

DISCUSSION

Raptor Use of Prairie Dog Colonies

We observed ferruginous hawks and golden eagles hunting and foraging at prairie dog colonies but did not observe them scavenging on shot prairie dogs. A sylvatic plague (*Yersinia pestis*) outbreak had drastically reduced prairie dog numbers at many of the colonies in TBNG during 2001-2002 (T. Byer, USFS, pers. comm. 10/31/01) and may explain, in part, why we did not observe any raptors scavenging on shooter-killed prairie dogs. Furthermore, raptor use did not increase as shooting intensity increased or as the colony size increased as we had predicted. This may be explained because there was an increase in the availability of alternate prey species (in addition to a reduction in the number of prairie dogs due to the sylvatic plague outbreak). Long-term surveys in this area indicated that Lagomorph numbers were increasing (Clayton 2001). Because Lagomorphs were relatively abundant, foraging and scavenging at prairie dog colonies may have been less likely. However, it is well documented that raptors will scavenge carcasses such as shooter-killed jackrabbits (Platt 1976), deer, and other animals (Craig et al. 1990; Bechard and Schmutz 1995). Further investigation may be warranted to determine if raptors are ingesting Pb fragments from shooter-killed prairie dogs once prairie dog populations rebound, Lagomorph populations decline, and the raptors return to an-almost-exclusive diet of shooter-killed prairie dogs.

Bullet Fragments in Prairie Dog Carcasses

We found Pb fragments in three of the prairie dog carcasses examined. Although it is not possible to assess if Pb toxicity would have occurred in the raptors at TBNG if those carcasses were scavenged, the results of the Pb fragments excised from the prairie dog carcasses, as well as the radiographs show that Pb in shooter-killed prairie dogs is available and the potential for ingestion by scavengers, including raptors, is present.

Factors that can affect the toxicity of Pb if ingested by raptors include the health of the bird at the time of ingestion, amount and frequency of Pb ingestion, and whether Pb pieces are ingested or regurgitated as a casting. Miller et al. (2000) reported that although 9% of golden eagles and bald eagles sampled in Saskatchewan, Canada ingested shotshell pellets from scavenging dead waterfowl, regurgitated castings (<2%) contained Pb shotshell pellets. However, Redig et al. (1980) found that Pb fragments that are consumed are not necessarily

incorporated into regurgitated castings and can be retained in the gastrointestinal tract resulting in Pb poisoning. In particular, bald eagles do not regularly regurgitate castings, which can result in longer retention of Pb pellets and more susceptibility to Pb poisoning (Redig et al. 1980).

Furthermore, adult raptors feed their nestlings the prime tissues of scavenged carcasses (Pattee 1984), which tend to contain the greater amount of shot, potentially exposing the young to greater amounts of Pb. In addition to this increased risk, poisoning caused by ingestion of food items containing Pb has greater impacts on nestlings of various altricial birds, such as American kestrels, golden eagles and ferruginous hawks, than adults (Hoffman et al. 1985b). Such impacts on nestlings include mortality, reduced growth, and pronounced hematological effects (Hoffman et al. 1985b). We did not have any nestlings that succumbed to the toxic effects of Pb and we do not know for certain if any of the nestlings we observed actually ingested Pb, but as little as one Pb shot in waterfowl, if ingested, can cause acute or chronic toxicity effects, with chronic effects not often noticeable at first (Wobeser 1981).

Based on our recovery and analysis of bullet fragments from four of the ten carcasses, copper appeared to be the metal most consumed by scavenging raptors. Approximately 113 mg, 10 mg, and 67 mg of copper were found in three carcasses. However, we did not analyze copper concentrations in blood and found little information in our literature review of copper toxicity in raptors. More research is needed on copper poisoning in raptors.

Blood and Feather Analysis

Blood Pb concentrations in all ferruginous hawks and golden eagles sampled were well below sub-clinical levels of 0.2 to 1.5 $\mu\text{g Pb/g}$ wet weight (Franson 1996) except for the occasional outlier. ‘Sub-clinical’ is indicative of potential physiological injury from which the bird would probably recover if Pb exposure was terminated (Franson 1996). The levels that we detected indicate Pb is present in the environment but at low levels. Additionally, comparisons of ALAD activity, protoporphyrin levels, and hemoglobin levels between ferruginous hawks at control and treatment sites indicate that Pb poisoning was not occurring in raptors in TBNG. However, PCV of nestlings at TBNG was significantly lower than at the control site. It is likely that PCV was lower at TBNG because of nestling age differences. The average age of nestlings sampled in Rawlins and TBNG was 39.2 and 26.5 days old, respectively. PCV increases in young animals with age to fulfill the increasing metabolic demands for oxygen associated with increasing body size (Rawson et al. 1992).

Analysis of nestling ferruginous hawk and golden eagle feathers for Pb concentrations was conducted with the purpose of having an additional measure of Pb poisoning for this study. According to Burger (1993), concentrations of metals incorporated into feathers reflect concentrations of metals in the blood at the time of feather formation. The metals concentrations therefore, represent current dietary exposure and metals mobilized from internal organs. For juvenile birds that have not undergone a migration, the metal concentrations in their feathers represent the exposure to metals at a given location (Burger 1993; Golden et al. 2003).

However, some feather Pb concentrations in our samples were much higher than others, which did not correlate with the respective blood analyses. Examples of this include: 1) a golden eagle that had a feather Pb concentration of 1,070 $\mu\text{g Pb/g}$ and a blood Pb concentration of only 0.035 $\mu\text{g Pb/g}$ and 2) a ferruginous hawk that had a feather Pb concentration of 24.7 $\mu\text{g Pb/g}$ and a blood Pb concentration of only 0.023 $\mu\text{g Pb/g}$. Burger and Gochfeld (2000) reported that tissue type is the strongest contributor responsible for the variation in concentrations of metals, with Pb concentrations highest in feathers. Even though Pb concentrations are known to be highest in feathers, we were unsure how to interpret such large discrepancies. Additionally, there were no reference values for Pb concentration in feathers of Falconiformes available in the scientific literature to indicate a Pb poisoning threshold; although, metal levels in blood represent short-term exposure while feather metal levels represent longer, chronic body burdens (during the last molt) (Evers et al. 2005).

If Pb poisoning was occurring in raptors at TBNG from scavenging shot prairie dogs, there are several reasons why it was undetectable. First, the plague severely reduced prairie dog numbers in 2001-2002. Second, 13% of TBNG was closed to prairie dog shooting in an effort to reintroduce black-footed ferrets. Increased regulations on shooting and decreased numbers of prairie dogs contributed to a decrease in the number of shooters visiting TBNG during the course of our study. Finally, long-term surveys in this area indicate that Lagomorphs were very abundant, thus, the likelihood of birds of prey scavenging shot prairie dogs during our study may have been reduced.

Even though we did not detect Pb poisoning at TBNG, our results confirm that some carcasses of shot prairie dogs contain Pb fragments and scavenging could result in Pb poisoning. The frequency with which it occurs is likely to be uncommon and dependent on other factors such as prairie dog numbers, shooter intensity, availability of alternate food sources, and regulations on shooting.

A repeat of this unique study would be beneficial in determining if an increase in shooting pressure on prairie dogs would result in an increase in the abundance of raptors feeding on prairie dog carrion and subsequently an increase in blood Pb concentrations in the raptors. Golden eagle populations in Wyoming are declining (Wyoming Game and Fish Dept. 1995) and the ferruginous hawk is considered a sensitive species by the USFS. The bald eagle may be delisted from the Threatened and Endangered Species list in the near future but numerous threats still remain to these birds including Pb poisoning. Additionally, a more in-depth study would allow us to collect data on potential impacts to raptors associated with the ingestion of copper fragments embedded in shooter-killed prairie dogs. Obtaining additional feather samples from juvenile raptors would be beneficial for examining differences in metal concentrations between pin feathers and down feathers. Correlations between metal concentrations in blood and feathers would be better defined with additional sampling of raptors at TBNG. Sampling both juvenile and adult ferruginous hawks, golden eagles, and burrowing owls would be beneficial for determining differences in metal concentrations among species and to determine if age-related differences exist in the accumulation of Pb.

Acknowledgments—The authors thank the U.S. Fish and Wildlife Service, Wyoming Game and Fish Department, and Wyoming Cooperative Fish and Wildlife Research Unit for funding this research. Additionally, the following individuals made significant contributions to this study: L. Apple, T. Byer, K. Clayton, C. J. Boese, C. Franson, K. Gordon, K. Keffer, S. Lantz, C. Lockman, J. S. Meyer, M. Raisbeck, and R. Siemion. Finally, we thank K. Munney and D. Major of the U.S. Fish and Wildlife Service, J. Meyer of the University of Wyoming, and two anonymous reviewers for their comments in improving the manuscript. Capture and handling protocols were reviewed and approved under the University of Wyoming Animal Care and Use Committee Form number A-3216-01.

REFERENCES

- Bechard, M.J. and J.K. Schmutz. 1995. Ferruginous hawk (*Buteo regalis*). In: A. Poole and F. Gill, eds. *The Birds of North America, No. 172*. The Academy of Natural Sciences, Philadelphia, and The American Ornithologists' Union, Washington, D.C. 20 pp.
- Benson, W.W., B. Pharaoh, and P. Miller. 1974. Lead poisoning in a bird of prey. *Bull. Environ. Contam. Toxicol.* 11: 105-108.
- Burch, H.B. and A.L. Siegel. 1971. Improved method for measurement of delta-aminolevulinic acid dehydratase activity of human erythrocytes. *Clin. Chem.* 17: 1038-1041.
- Burger, J. 1993. Metals in avian feathers: Bioindicators of environmental pollution. *Rev. Environ. Toxicol.* 5: 203-311.
- Burger, J. and M. Gochfeld. 2000. Metals in Laysan albatrosses from Midway Atoll. *Arch. Environ. Contam. Toxicol.* 38: 254-259.
- Clayton, K.M. 2001. Raptor ecology in the Thunder Basin of northeast Wyoming. Thunder Basin Grasslands Prairie Ecosystem Association Technical Bulletin Number 1: The History, Ecology, and Economy of the Thunder Basin Prairie Grasslands: Building a knowledge base for an ecosystem management plan. Douglas, WY.
- Craig, T.H., J.W. Connelly, E.H. Craig, and T.L. Parker. 1990. Lead concentrations in golden and bald eagles. *Wilson Bull.* 102: 130-133.
- Evers, D.C., N.M. Burgess, L. Champoux, B. Hoskins, A. Major, W. Goodale, R. Taylor, R. Poppenga, and T. Daigle. 2005. Patterns and interpretation on mercury exposure in freshwater avian communities in northeastern North America. *Ecotox.* 14: 193-221.
- Fernandez, F.J. and D. Hilligoss. 1982. Improved graphite furnace method for determination of lead in blood using matrix modification and the L'vov platform. *Atom. Spec.* 3: 130-131.

- Franson, C.J. 1986. Immunosuppressive effects of lead. In: *Lead Poisoning in Wild Waterfowl: A Workshop*, J.S. Feierabend and A.B. Russell, eds. National Wildlife Federation. Washington D.C. Pp. 106-109.
- Franson, J.C. 1996. Interpretation of tissue lead residues in birds other than waterfowl. In: W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood, eds. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. CRC Press, Inc. Boca Raton, Florida. pp. 265-279.
- Franson, J.C., W.L. Hohman, J.L. Moore, and M.R. Smith. 1996. Efficacy of protoporphyrin as a predictive marker for lead exposure in canvasback ducks: Effect of sample storage time. *Environ. Monit. Assess.* 43: 181-188.
- Franson, J.C., L. Sileo, O.H. Pattee, and J.F. Moore. 1983. Effects of chronic dietary lead in American kestrels (*Falco sparverius*). *J. Wildl. Diseases.* 19: 110-113.
- Golden, N.H., B.A. Rattner, J.B. Cohen, D.J. Hoffman, E. Russek-Cohen, and M.A. Ottinger. 2003. Lead accumulation in feathers of nestling black-crowned night herons (*Nycticorax nycticorax*) experimentally treated in the field. *Environ. Toxicol. Chem.* 22: 1515-1524.
- Henny C.J., L.J. Blus, D.J. Hoffman, L. Sileo, D.J. Audet, and M.R. Snyder. 2000. Field evaluation of lead effects on Canada geese and mallards in the Coeur d'Alene River Basin, Idaho. *Arch. Environ. Contam. Toxicol.* 39: 97-112.
- Hoffman, D.J., J.C. Franson, O.H. Pattee, C.M. Bunck, and A. Allen. 1985a. Survival, growth, and accumulation of ingested lead in nestling American kestrels (*Falco sparverius*). *Arch. Environ. Contam. Toxicol.* 14: 89-94.
- Hoffman, D.J., J.C. Franson, O.H. Pattee, C.M. Bunck, and H.C. Murray. 1985b. Biochemical and hematological effects of lead ingestion in nestling American kestrels (*Falco sparverius*). *Comp. Biochem. Physiol.* 80C: 431-439.
- Jacobson, E., J.W. Carpenter, and M. Novilla. 1977. Suspected lead toxicosis in a bald eagle. *J. Am. Vet. Med. Assoc.* 171: 952-954.
- Leonzio, C. and M.C. Fossi. 1994. Nondestructive biomarker strategy: Perspectives and applications. In: M.C. Fossi and C. Leonzio, eds. *Nondestructive Biomarkers in Vertebrates*. CRC Press, Inc. Boca Raton, Florida. pp. 297-312.
- Locke, L.N., G.E. Bagley, D.N. Frickie, and L.T. Young. 1969. Lead poisoning and aspergillosis in an Andean condor. *J. Am. Vet. Med. Assoc.* 155: 1052-1056.

- Mayer, F.L., D.J. Versteeg, M.J. McKee, L.C. Folmar, R.L. Graney, D.C. McCume, and B.A. Rattner. 1992. Physiological and nonspecific biomarkers. In: R.J. Huggett, R.A. Kimerle, P.M. Mehrl, Jr., and H.L. Bergman, eds. *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress*. Lewis Publishers. Chelsea, Michigan. pp.5-85.
- Miller, M.J.R., M.E. Wayland, E.H. Dzus, and G.R. Bortolotti. 2000. Availability and ingestion of lead shotshell pellets by migrant bald eagles in Saskatchewan. *J. Raptor Res.* 34: 167-174.
- Pain, D.J. 1995. Lead in the environment. In: D.J. Hoffman, B.A. Rattner, G.A. Burton, Jr., and J. Cairns, Jr., eds. *Handbook of Ecotoxicology*. Lewis Publishers, Boca Raton, Florida. pp.356-391.
- Pain, D.J. 1996. Lead in waterfowl. In: W.N. Beyer, G.H. Heinz, and A.W. Redmon-Norwood, eds. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. CRC Press, Inc. Boca Raton, Florida. pp. 265-279.
- Pattee, O.H. 1984. Eggshell thickness and reproduction in American kestrels exposed to chronic dietary lead. *Arch. Environ. Contam. Toxicol.* 13: 29-34.
- Pattee, O.H., S.N. Wiemeyer, B.M. Mulhern, L. Sileo, and J.W. Carpenter. 1981. Experimental lead-shot poisoning in bald eagles. *J Wildl. Manage.* 45: 1981.
- Platt, J.B. 1976. Bald eagles wintering in the Utah desert. *Am. Birds.* 30: 783-788.
- Rawson, R.E., G.D. DelGiudice, H.E. Dziuk, and L.D. Mech. 1992. Energy metabolism and hematology of white-tailed deer fawns. *J. Wildl. Dis.* 28: 91-94.
- Redig, P.T., C.M. Stowe, D.M. Barnes, and T.D. Arent. 1980. Lead toxicosis in raptors. *J. Am. Vet. Med. Assoc.* 177: 941-943.
- Sari M., S. de Pee, E. Martini, S. Herman, W. Sugiatmi, M. Bloem, and R. Yip. 2001. Estimating the prevalence of anemia: A comparison of three methods. *Bull. World Health Org.* 79: 506-511.
- United States Fish and Wildlife Service. 2000. *Fish and Wildlife News*. September/October 2000. Washington D.C. Pg. 6.
- United States Fish and Wildlife Service. 1996. *Standard Operating Procedures for Environmental Contaminants Operations*. Volume 1. Department of Interior, Washington D.C.
- United States Fish and Wildlife Service. 1986. *Use of lead shot for hunting migratory birds in the United States*. Final supplemental environmental impact statement. Department of Interior, Washington D.C.

United States Forest Service and United States Bureau of Land Management. 1992. Oil and gas leasing on the Thunder Basin National Grassland. Final Environmental Impact Statement. Douglas Ranger District. Various pages.

Wobeser, G.A. 1981. *Diseases of Wild Waterfowl*. Plenum Press, New York. 300 pp.

Wyoming Game and Fish Department. 1995. Endangered and non-game bird and mammal investigations. 1995 Annual Completion Report. Cheyenne, Wyoming.