esa ECOSPHERE

AGROECOSYSTEMS

The lead (Pb) lining of agriculture-related subsidies: enhanced Golden Eagle growth rates tempered by Pb exposure

Garth Herring,[1] Collin A. Eagles-Smith,[1,†] Jeremy A. Buck,[2] Alyssa E. Shiel,[3] Chris R. Vennum,[4] Colleen Emery,[1] Branden Johnson,[1] David Leal,[2] Julie A. Heath,[5] Benjamin M. Dudek,[5] Charles R. Preston,[6] and Brian Woodbridge[7]

[1]*Forest and Rangeland Ecosystem Science Center, U.S. Geological Survey, Corvallis, Oregon 97331 USA*
[2]*Oregon Fish and Wildlife Office, U.S. Fish and Wildlife Service, Portland, Oregon 97266 USA*
[3]*College of Earth, Ocean, and Atmospheric Sciences, Oregon State University, Corvallis, Oregon 97331 USA*
[4]*University of Nevada, Reno, Nevada 89557 USA*
[5]*Boise State University, Boise, Idaho 83725 USA*
[6]*Draper Natural History Museum, Buffalo Bill Center of the West, Cody, Wyoming 82414 USA*
[7]*Division of Migratory Bird Management, U.S. Fish and Wildlife Service, Corvallis, Oregon 97330 USA*

Citation: Herring, G., C. A. Eagles-Smith, J. A. Buck, A. E. Shiel, C. R. Vennum, C. Emery, B. Johnson, D. Leal, J. A. Heath, B. M. Dudek, C. R. Preston, and B. Woodbridge. 2020. The lead (Pb) lining of agriculture-related subsidies: enhanced Golden Eagle growth rates tempered by Pb exposure. Ecosphere 11(1):e03006. 10.1002/ecs2.3006

Abstract. Supplementary food resources (e.g., subsidies) associated with agriculture can benefit wildlife species, increasing predictability and availability of food. Avian scavengers including raptors often utilize subsidies associated with both recreational hunting and pest shooting on agricultural lands. However, these subsidies can contain lead (Pb) fragments if they are culled with Pb-based ammunition, potentially leading to Pb poisoning and physiological impairment in wildlife. Nesting Golden Eagles (*Aquila chrysaetos*) commonly forage in agricultural lands during the breeding season, and therefore, both adults and their nestlings are susceptible to Pb exposure from scavenging shot wildlife. We assessed drivers of Pb exposure in 258 nestling Golden Eagles (401 total blood samples), along with physiological and growth responses, in agricultural lands across four western states in the United States. We also evaluated the birds' Pb stable isotope signatures to inform exposure sources. Twenty-six percent of Golden Eagle nestlings contained Pb concentrations associated with subclinical poisoning for sensitive species (0.03–0.2 μg/g ww), 4% had Pb concentrations that exceeded subclinical poisoning benchmarks (0.2–0.5 μg/g ww), and <1% exceeded either concentrations associated with clinical poisoning (0.5–1.0 μg/g ww) and or those deemed to cause severe clinical poisoning (>1.0 μg/g ww). Lead concentrations were highest in nestlings with close proximity to fields that potentially provided subsidies and declined exponentially as distance to subsidies increased. However, close proximity to agriculture, and presumably subsidies, positively influenced nestling growth rates. Across the range of Pb exposure, nestlings experienced a 67% reduction in delta-aminolevulinic acid dehydratase (δ-ALAD) activity, suggesting nestlings may have been anemic or experiencing cellular damage. Isotopic ratios of $^{206}Pb/^{207}Pb$ increased non-linearly with increasing blood Pb in Golden Eagle nestlings, and 45% of the birds were consistent with those of ammunition. However, above 0.10 μg/g ww, the proportion associated with ammunition increased to 89% of the nestlings. An improved understanding of how these positive (growth) and negative (physiology) effects associated with proximity to subsidies interact would be beneficial to managers when considering management scenarios and potentially evaluating any measures taken to reduce Pb exposure across the landscape.

Key words: agricultural pest management; *Aquila chrysaetos;* bullet fragments; delta-aminolevulinic acid dehydratase; ecotoxicology; lead exposure; lead isotopes; recreational shooting; western United States.

Received 26 July 2019; revised 25 October 2019; accepted 7 November 2019. Corresponding Editor: Paige S. Warren.

† **E-mail:** ceagles-smith@usgs.gov

Introduction

Landscape conversion to agriculture degrades existing habitat quality and impacts ecosystem services globally (Hoekstra et al. 2004, Haines-Young 2009, Seoraj-Pillai and Pillay 2017). Alteration of wildlife habitat is a particularly common result of agricultural development (Seoraj-Pillai and Pillay 2017), but net effects to wildlife can be complex, resulting in both positive (Wolff et al. 2001, Cardador et al. 2011, Murgatroyd et al. 2016) and negative (Arroyo et al. 2002, Costantini et al. 2014, Almasi et al. 2015) responses. Benefits of agriculture to wildlife populations can occur through access to supplementary food resources (hereafter subsidies) in a previously low-productivity environment (Cortés-Avizanda et al. 2012, Oro et al. 2013), where the temporal and spatial predictability (and availability based on land management practices) of these subsidies improves access to food resources compared to natural systems (Bartumeus et al. 2010, Cortés-Avizanda et al. 2012). As a result, subsidies can decrease time spent foraging and improve physiological condition and breeding performance (Robb et al. 2008, Oro et al. 2013). However, agricultural subsidies can also result in potential ecological traps where wildlife select maladaptive habitats that may result in physiological impairment (Fritsch et al. 2019) or population effects (Battin 2004, Hale and Swearer 2016).

Raptor species are good indicators of ecosystem health because they are often apex predators and are vulnerable to modification of the landscapes they occupy (Sergio et al. 2006, Terraube and Bretagnolle 2018). Although agriculture-induced habitat changes can have negative consequences to raptor populations (Arroyo et al. 2002, Costantini et al. 2014, Almasi et al. 2015), agriculture can also provide benefits associated with increased availability of subsidies such as increased abundance or availability of prey resources (Panek and Hušek 2014, Murgatroyd et al. 2016). In particular, scavenging raptor species respond positively to predictable subsidies such as offal and carrion associated with agricultural or hunting activities (Wilmers and Getz 2004, Monsarrat et al. 2013). Where agricultural practices intersect with hunting and pest management activity, there is potential for increased availability of carcasses for scavenging raptors (Harmata and Restani 1995, Stephens et al. 2008, Herring et al. 2016, McTee et al. 2017, 2019). Despite the positive benefits from subsidies, they also portend risks to raptors resulting from exposure to anthropogenic contaminants (Walters et al. 2008), particularly lead (Pb; Haig et al. 2014, Naidoo et al. 2018).

Globally, Pb is a major threat to scavenging raptors that feed on hunter-killed offal, and unretrieved game and managed pest carcasses that have been shot with Pb-based ammunition (Haig et al. 2014, Ecke 2017, Garbett et al. 2018, Gil-Sánchez et al. 2018). As one of the largest predatory and scavenging species of raptors, Golden Eagles (*Aquila chrysaetos*) are at risk of substantial Pb exposure from scavenging throughout their global range (Austria, Kenntner et al. 2007; Canada, Wayland and Bollinger 1999; Germany, Kenntner et al. 2007; Italy, Squadrone et al. 2018; Japan, Ishii et al. 2017; Sweden, Ecke 2017; Switzerland, Jenni et al. 2015; USA, Langner et al. 2015). Golden Eagles often breed and forage in proximity to agricultural environments (Craig and Craig 1984, Menkens and Anderson 1987) and are attracted to hunter-killed offal and recreational shot and agriculture pest management subsidies (Harmata and Restani 1995, Legagneux et al. 2014, Herring et al. 2017).

Within North America, Pb exposure in Golden Eagles is well documented; however, most of the available information has been derived from free-ranging (migrating or breeding) adult birds (Pattee et al. 1990, Stauber et al. 2010, Langner et al. 2015, Watson and Davies 2015). In contrast, Pb exposure in Golden Eagle nestlings is relatively understudied despite evidence suggesting that early developmental stages are the most critical periods for exposure to environmental contaminants, in particular Pb (Hoffman et al. 1985, Epsín et al. 2015, Herring et al. 2017). Further, little is known about how local subsidies associated with recreational shooting/hunting and pest management shooting on or near agricultural lands during the breeding season influence nestling health or Pb exposure. To determine the extent of Pb exposure in Golden Eagle nestlings in the western United States associated with agriculture and pest shooting (e.g., ground squirrels Sciuridae, coyotes *Canis latrans*) and potential influence of shot subsidies, we sampled nestlings in four western states, examining both biological and landscape factors that influenced Pb exposure and nestling growth rates. Additionally, we evaluated a common biomarker of Pb exposure (delta-aminolevulinic acid dehydratase [δ-ALAD], a precursor to impaired heme synthesis) to determine if there were associated physiological effects and sought to determine potential sources of Pb exposure using Pb stable isotope ratios.

Methods

Study area

We sampled Golden Eagle nestlings at four locations across the western United States, including Butte Valley in northern California, Northern Great Basin in central and eastern Oregon, the Morley Nelson Snake River Birds of Prey National Conservation Area and surrounding regions in southern Idaho, and the Bighorn Basin in Wyoming (Fig. 1). Nest sites were a mixture of cliffs (81%) and trees (19%), with tree nests being in either cottonwood (*Populus*), juniper (*Juniperus*), or pine (*Pinus*) trees. Detailed descriptions of the study areas and diet of Golden Eagles can be found in Bedrosian et al. (2017) and Preston et al. (2017). During April to June, 2013–2016, we sampled 258 individual Golden Eagle nestlings from 156 individual nests for a total of 401 blood samples. When possible, we sampled blood from each individual nestling twice: The first sampling occurred at 14–51 d post-hatch and the second sampling occurred at 27–61 d after hatch development, because blood Pb concentrations can be dynamic over time. We collected blood (<1% of body mass) from the brachial vein using 25- to 27-gauge heparinized needles and syringe, or a butterfly needle and ethylenediaminetetraacetic acid (EDTA) vacuum collection tubes, and stored blood on ice until freezing at −80°C. An aliquot of blood was taken was taken to determine hematocrit for δ-ALAD measurements.

During the first nest entry, nestlings were banded with a stainless-steel U.S. Geological Survey leg band or were uniquely marked for subsequent identification and later banded if nestlings were too small to retain a band. During each nest entry, we recorded mass to the nearest 100 g using a Pesola spring scale (Pesola AG, Schindellegi, Switzerland) and estimated age based on feather development, following Hoechlin (1976) and Driscoll (2010). Given the variation in development of nestlings during visits, we could not definitively assign hatch order or sex to individuals.

Blood Pb determination

Blood samples were digested using concentrated, ultra-pure nitric acid following Andersen (1996), filtered through a 45-μm polyvinylidene difluoride filter, and spiked with an internal standard (indium). We determined Pb concentrations of digested blood samples using a Thermo Scientific X-Series II CCT ICPMS (Thermo Fisher Scientific, Waltham, Massachusetts, USA). External calibration curves were prepared in aqueous solutions using a commercial Pb standard (Ricca Chemical Company, Arlington, Texas, USA; PPB IKN-100). Certified reference materials (blood; National Institute of Standards and Technology SRM 955c), method blanks, and duplicates were used for method validation. Lead recovery averaged 98.6% ± 4.7% for certified reference materials ($n = 14$), the absolute relative percent difference for duplicates averaged 8.2% ± 2.2% ($n = 18$), and the limit of detection for Pb was 0.002 μg/g. Some nestlings (16%) had blood Pb concentrations below the limit of detection, and we used the method of median semi-variance (SemiV) to estimate values for the left-censored data as described by Zoffoli et al. (2013). The SemiV approach to handling left-censored data minimizes the bias in associated error for the mean and standard deviation (Zoffoli et al. 2013) and is not flawed like traditional substitution and deletion methods that have no statistical underpinning (Helsel 2006, Zoffoli et al. 2013). We present blood Pb concentrations as μg/g (parts per million; ppm) wet weight. Lead concentrations are often reported in μg/dL, which can be estimated by multiplying μg/g concentrations by 100.

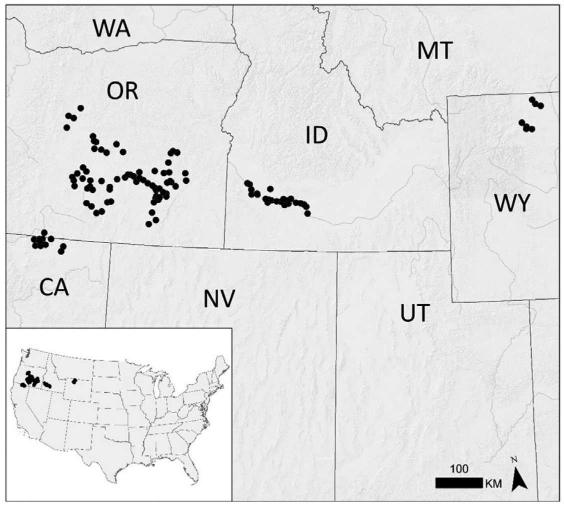

Fig. 1. Nest site locations of Golden Eagle (*Aquila chrysaetos*) nestlings sampled in California (CA), Idaho (ID), Oregon (OR), and Wyoming (WY) during 2013–2016.

Blood Pb isotopes

Pb stable isotopes can be used as tracers of environmental pollution with ratios of the individual Pb isotopes reflecting potential sources (Komárek et al. 2008, Finkelstein et al. 2012, Legagneux et al. 2014, Sriram et al. 2018). To understand potential sources of Pb exposure in Golden Eagle nestlings, we randomly selected a subset of blood samples for analysis of Pb stable isotope ratios that spanned the entire range of Pb exposure we previously identified in the Pb-analysis stage. To ensure independence among samples, we only analyzed Pb ratios from one nestling per nest. We procured donated unfired rounds of ammunition that are commonly used throughout our study area for either recreational ground squirrel shooting or agriculture pest management shooting to estimate the $^{206}Pb/^{207}Pb$ isotope ratios of Pb-based ammunition; Federal American Eagle .223 (Federal Ammunition, Anoka Minnesota, USA), Federal Sierra .308, and Remington Core Lokt 30-06 (Remington Arms Company, Madison, North Carolina, USA). Additionally, we recovered known ammunition type fragments from shot ground squirrels from two regions of our study in California and Oregon (Herring et al. 2016); Hornady .17 HMR V-Max (Hornady Manufacturing Company, Grand Island, Nebraska, USA), Hornady .17 Win Super Mag, Remington Thunderbolt .22 solid, and CCI .22 hollow point (CCI Ammunition, Lewiston, Idaho, USA).

All Pb isotope work was conducted in a Class 100 clean laboratory at the Keck Collaboratory for Plasma Spectrometry, Oregon State University. Blood samples were digested using successive treatments of concentrated, ultra-pure nitric acid. Bullet fragments were leached with 1 mol/L HCl and resulting leachates were collected to represent the bullets. Sample Pb was isolated by anion exchange chromatography using the AG 1-X8 (100–200 mesh) resin (Bio-Rad Laboratories, Hercules, California, USA) as previously described (Weis et al. 2006). Lead isotopes were measured using the Nu Plasma 023 (Nu Instruments, Charlestown, Massachusetts, USA) multi-collector inductively coupled plasma mass spectrometer. Samples and standards were prepared in 3% nitric acid and introduced into the instrument with an Aridus II desolvating nebulizer system (CETAC Technologies, Omaha, Nebraska, USA). Ion signal intensities were measured for masses 202 through 208 (isotopes of Pb and Hg). We used the NIST (USA) SRM 981 natural Pb (isotopic) standard for monitoring analytical run instrument drift and normalization of all measured Pb isotopic ratios. At the start of each analytical session a batch (≥3) of the NIST SRM 981 standard was run. Samples were run following a modified sample-standard bracketing measurement protocol, where the standard was run after every two samples. A minimum of two USGS-certified reference materials, BCR-1 (basalt; $^{206}Pb/^{207}Pb$ certified ratio = 1.2099) and AGV-1 (andesite; $^{206}Pb/^{207}Pb$ certified ratio = 1.2037), were run during each analytical session for quality assurance (Weis et al. 2006). Certified reference material $^{206}Pb/^{207}Pb$ ratios (± SD) averaged 1.2100 ± 0.0005 (BCR-1; $n = 6$) and 1.2038 ± 0.0006 (AGV-1; $n = 8$) across all analytical sessions.

Delta-aminolevulinic acid dehydratase

Delta-aminolevulinic acid dehydratase activity is a precursor to heme synthesis and a sensitive indicator of physiological response to Pb exposure (Epsín et al. 2015). After identifying the range of Pb exposure, we randomly selected a subset of blood samples for δ-ALAD determination that spanned that full range. We measured δ-ALAD activity using a variation of the European standard method (Berlin and Schaller 1974). We mixed 100 μL of whole blood with 1.5 mL nanopure water in a 5-mL centrifuge tube and vortexed at 2000 rpm for 10 s. We added 1 mL of 10 μmol/L ALA solution (Sigma, St. Louis, Missouri, USA), vortexed for 10 s, and incubated in the dark at 38°C for 60 min. One mL of trichloroacetic acid stop solution (10%) was added to stop the reaction, followed by 10-second vortex. We then centrifuged tubes for 10 min at 2000 × g and a 100-μL aliquot in duplicate was removed and transferred to a 96-well plate. Ehrlich's indicator reagent (100 μL) was added and plates were read on a VERSAmax (Molecular Devices, Sunnyvale, California, USA) microplate reader at 555 nm. Delta-aminolevulinic acid dehydratase activity is presented as $nmol \cdot min^{-1} \cdot mL^{-1}$ whole blood. Absolute relative percent difference for duplicates averaged 2.1% ± 0.9%.

Landscape analysis

To understand the potential influence of agricultural subsidies on Golden Eagle nestling Pb exposure and growth rates, we used ArcMap 10.4.1 (ESRI, Redlands, California, USA) to quantify a suite of landscape variables associated with each nest site. We first calculated the land area (km^2) within the core area of the nest where a sample was collected (4.7 km + 1 standard error [SE]; Marzluff et al. 1997) that was comprised of hay (primarily alfalfa; *Medicago sativa*) agriculture (hereafter core subsidy area) using the U.S. Department of Agriculture's National Agricultural Statistics Service; Crop Scape and Cropland Data Layers (U.S. Department of Agriculture 2016). Although multiple crop types existed within the core area of some nests, we focused our analysis on hay crops because they can be a source of important Golden Eagle prey resources (Bedrosian et al. 2017, Herring et al. 2016, 2017), are commonly sites of intensive recreational and pest management shooting activity which provide subsidies (Herring et al. 2016, McTee et al. 2017, 2019), and are the most common crop across the landscape studied (72%). Core subsidy area served as a proxy for potential abundance of agricultural-associated prey and we assumed that prey abundance and/or availability increased with core subsidy area. We then measured the distance (km) from each nest to the nearest hay field (hereafter distance to subsidy) using the CropScape and Cropland Data Layers. Distance to subsidy served as a measurement of subsidy resource access. We assumed that adult Golden Eagle nests that were closer to hay fields would have increased access to shot subsidies. Because there were annual changes in which fields were farmed, as well as their boundaries, we used year-specific layers for each nest that was associated with the year in which it was sampled.

Data analysis

We used an information-theoretic approach (Burnham and Anderson 2002) and linear mixed models in R (LME4; R Development Core Team, 2011) to determine the variables that most influenced Pb concentrations and growth rates in Golden Eagle nestlings. We built and ranked separate competing candidate models to understand how Pb concentrations in each bird were influenced by differing biological and landscape variables. We included all possible variable combinations to ensure a balanced design, which facilitates a comparison of variable weights, and included an intercept only (null) model and global model. Where appropriate we included linear, quadratic, and cubic forms of variables (e.g., age; Herring et al. 2013) in preliminary models, but only retained the linear forms in the final model set as they always had the most support. When examining factors influencing nestling Pb exposure, we included the following variables in our model: nestling age (days post-hatch based on plumage estimates), distance from nest to nearest subsidy (km), and core subsidy area (km^2). Prior to analysis we evaluated correlations between all parameters included in modeling efforts; we found no signs of correlations between model parameters (all $r \leq 0.19$). We also included a unique identifier for each nestling and nest within each year as random effects to account for the repeated sampling of nestlings, and the non-independence of sampling multiple nestlings within a nest. Similarly, we included region as a main effect in all models, to account for potential differences in diet across the landscape. In some cases, the same territory or nest was sampled in multiple years, but we treated each year as an independent sample. We then used Akaike's information criterion adjusted for small samples sizes (AIC_c) to evaluate the fit of the data to each model and considered the model with the lowest AIC_c value to be the most parsimonious (Burnham and Anderson 2002). We ran all possible models and considered candidate models with $\Delta AIC_c \leq 2.0$ to be equally plausible and models with $\Delta AIC_c > 4.0$ to have less support. We determined the relative ranking of each model by subtracting each candidate model's AIC_c value from the best model (ΔAIC_c). We present all candidate models with $\Delta AIC_c \leq 4.0$ and the null model in Tables 1, 2. We calculated Akaike weights to assess the weight of evidence for candidate models (Burnham and Anderson 2002), and calculated cumulative variable weights by summing Akaike weights across all models that included the variable to assess the relative importance of each variable. We calculated model-averaged beta coefficient estimates from all candidate models (Burnham and Anderson 2002) and present them with 85% confidence intervals to avoid variable selection uncertainty (Arnold 2010). We evaluated all possible two-way interactions across all models and did not include them in the final model set because they lacked support.

We used the same model selection procedures as above to examine how biological and landscape variables influenced nestling growth rates. We first calculated growth rates for individuals where we had nestling mass data from two consecutive visits by subtracting mass at nest entry 2 from mass at nest entry 1, then dividing the difference in mass by the number of days between nest entry 1 and nest entry 2. Because Golden Eagle nestlings follow a logistic growth curve (Collopy 1986) we only used data from the linear portion of the relationship between growth rate and age (10–50 d). Our initial models indicated that distance to subsidy was an important factor influencing Pb exposure (see *Results*) and therefore could not include both distance to subsidy and Pb concentration together in the same growth rate model. Thus, we used a two-stage approach for determining which variable had more support for influencing nestling growth rates. We first ran separate models with either distance to subsidy or Pb concentration as the independent variables and growth rate as the dependent variable and compared the AIC_c values from each model. The AIC_c value for distance to subsidy ($AIC_c = 1124.81$) was 7.63 AIC_c units lower than Pb concentration ($AIC_c = 1132.44$), demonstrating that the model with distance to subsidy had more support for explaining variation in nestling growth rates than Pb concentrations. Therefore, we retained distance to subsidy in subsequent nestling growth models and excluded Pb concentrations. We then ran the full model set that included distance to subsidy and core subsidy area as fixed effects. As in the previous models, we also included nest ID as a random effect to account for the non-independence of sampling multiple nestlings within a nest and region in every model to account for potential differences across regions.

We examined the relationship between nestling blood Pb concentrations and δ-ALAD activity by fitting an exponential model (Finkelstein et al. 2012) with δ-ALAD activity as the dependent variable and Pb concentration as the independent variable.

To understand the relationship between blood Pb concentrations and $^{206}Pb/^{207}Pb$ isotope ratios, we fit non-linear models to the data, selecting the model that best fit the data while minimizing the number of model parameters to avoid over fitting. Finally, to understand the potential origins of Pb in nestling Golden Eagles, we compared blood $^{206}Pb/^{207}Pb$ isotope ratios from Golden Eagle nestlings to Pb-based bullets sampled in this study and from the literature (Tsuji et al. 2008, Finkelstien et al. 2012). We also included $^{206}Pb/^{207}Pb$ isotope ratios from additional potential background sources (gasoline, Flegal et al. 2010; paint, Jaeger et al. 1998, Finkelstein et al. 2012; regional ore processing Pb effluent, Shiel

Table 1. Ranking of candidate models describing variables influencing Pb concentrations in nestling Golden Eagles (n = 397) in the western United States.

Model structure	K	−2Log$L$	ΔAIC_c	w_i	Evidence ratio
Distance to subsidy	8	1319.58	0.00	0.46	1.00
Distance to subsidy + age	9	1319.00	1.51	0.22	2.13
Distance to subsidy + area of subsidy	9	1319.53	2.03	0.17	2.77
Distance to subsidy + age + area of core subsidy	10	1318.94	3.56	0.08	5.92
Intercept	7	1328.14	6.47	0.02	24.40

Notes: Differences were determined using Akaike's information criterion adjusted for sample size (ΔAIC_c), model likelihood (−2Log$L$), model weight (w_i), number of parameters (K), and the weight of the evidence that the top model is better than the selected model (evidence ratio). Models presented include only those that were within four AIC_c units of the top model (ΔAIC_c = 0) and the null (intercept) model.

Table 2. Ranking of candidate models describing variables influencing nestling Golden Eagle growth rates (n = 131) in the western United States.

Model structure	K	−2Log$L$	ΔAIC_c	w_i	Evidence ratio
Distance to subsidy	7	1105.63	0.00	0.70	1.00
Distance to subsidy + area of core subsidy	8	1105.62	2.28	0.22	3.13
Intercept	6	1113.95	6.09	0.03	20.69

Notes: Differences were determined using Akaike's information criterion adjusted for sample size (ΔAIC_c), model likelihood (−2Log$L$), model weight (w_i), number of parameters (K), and the weight of the evidence that the top model is better than the selected model (evidence ratio). Models presented include only those that were within four AIC_c units of the top model (ΔAIC_c = 0) and the null (intercept) model.

et al. 2010; soil, Reimann et al. 2011). All estimates of background Pb isotope sources used were the most recent and geographically like our study.

Results

Nestling Pb

During 2013 to 2016 we sampled 401 Golden Eagle nestlings from 156 unique nest and year combinations (Butte Valley = 60, Northern Great Basin = 214, Morley Nelson Snake River Birds of Prey National Conservation Area = 103, Bighorn Basin = 24). Because the number of nests with three nestlings were low (n = 4) we did not include the third nestling (i.e., the smallest) in our statistical analyses. However, concentrations from the third nestling are presented separately as a geometric mean and are included in our summary of Pb exposure thresholds. Across all years and regions, nestling blood Pb levels (μg/g ww) ranged from <0.001 to 1.093 μg/g ww (n = 397) and the geometric mean Pb (±SE) level was 0.015 ± 0.002 μg/g ww. The geometric mean Pb level in third nestlings was 0.009 ± 0.004 μg/g ww. Twenty-six percent of Golden Eagle nestlings contained Pb concentrations associated with subclinical poisoning for sensitive species (0.03–0.2 μg/g ww, n = 104; Martínez-López et al. 2004, Finkelstein et al. 2012, Epsín et al. 2015), Pb concentrations in 4% of nestlings exceeded subclinical poisoning benchmarks (0.2–0.5 μg/g ww, n = 16; Franson and Pain 2011), <1% exceeded values associated with clinical poisoning (0.5–1.0 μg/g ww, n = 1; Franson and Pain 2011), and <1% (n = 1) had concentrations deemed to cause severe clinical poisoning (>1.0 μg/g ww; Franson and Pain 2011).

Variables influencing nestling Pb

The top model explaining Pb concentrations in nestling Golden Eagles contained only distance to subsidy, with a model weight of 0.46 (Table 1). One additional model that included age and distance to subsidy had reasonable support (<2 AIC_c units; Table 1), but the weight of the evidence suggested the top model was 2.13 times more likely than the next competitive model (Table 1). Using variable weights to assess variable importance, we found that models containing distance to subsidy had a combined AIC_c weight of 0.92, whereas there was little evidence for the effects of nestling age (0.32) or core subsidy area (0.30). Nestling Pb concentrations declined exponentially with increasing distances to subsidies (Fig. 2), and there was a 65% reduction in Pb concentrations from the closest (0.03 km) to the furthest (10.23 km) subsidy field ($\beta = -0.229 \pm 0.157$–85% confidence interval). Eighteen percent of nestlings within 1 km of a subsidy field had Pb concentrations exceeding the 0.10 μg/g background threshold (Craighead and Bedrosian 2008, Finkelstein et al. 2012), whereas 5% of nestlings exceeded this benchmark between 1 and 2 km from a subsidy field, and only 1% of nestlings >2 km from subsidy fields exceeded the background threshold (Fig. 2).

Nestling growth rates

We found that the most parsimonious model explaining nestling growth rates contained only distance to subsidy, with a model weight of 0.70 (Table 2). No other models were found to be competitive (<2 AIC_c units; Table 2). Nestling growth rates were negatively correlated with distance to subsidy fields and declined by 67% across the observed range (0.03–7.79 km; $\beta = -7.112 \pm 4.849$; Fig. 3). The growth rates of nestlings 3 km or closer to subsidy fields averaged 48 ± 4 g/d, whereas those located farther than 3 km from subsidy grew at an average rate of only 34 ± 10 g/d.

Delta-aminolevulinic acid dehydratase

There was a negative exponential relationship between blood δ-ALAD activity and Pb concentrations (δ-ALAD activity = $0.31 + 4.80 \times e^{(-3.78*\mathrm{Pb}\ \mu g/g)}$, $P = 0.003$, $r^2 = 0.49$; Fig. 4).

Nestling Pb isotopes

The $^{206}Pb/^{207}Pb$ stable isotope ratios ranged from 1.166 to 1.230, spanning ratios associated with a range of sources, from historic gasoline to Pb-based ammunition. Additionally, variation in $^{206}Pb/^{207}Pb$ ratios was heterogeneous across the range of Pb exposure; below 0.10 μg/g ww the coefficient of variation (CV) in Pb stable isotope ratios was 17%, whereas the CV was less than half that (8%) at concentrations above 0.10 μg/g (Fig. 5). The $^{206}Pb/^{207}Pb$ ratios were related to blood Pb concentrations in a non-linear fashion ($^{206}Pb/^{207}Pb = 1.197 \times \mathrm{Pb}\ \mu g/g\ ww/(3.15^{E-05} + \mathrm{Pb}\ \mu g/g\ ww) + 0.064 \times \mathrm{Pb}\ \mu g/g\ ww$), $r^2 = 0.28$; Fig. 5). At the lowest Pb exposure levels, stable isotope ratios increased sharply with slight increases in Pb concentrations, and there was an inflection point at Pb concentrations of 0.01 μg/g, above which the slope decreased and Pb stable isotope ratios were less variable. Across all birds, 45% of blood Pb stable isotope ratios fell within the range associated with Pb-based ammunition from this ($^{206}Pb/^{207}Pb = 1.219 \pm 0.004$ standard error) and previous studies ($^{206}Pb/^{207}Pb = 1.221 \pm 0.002$; Finkelstein et al. 2012, 1.228 ± 0.002; Tsuji et al. 2008), 31% of stable Pb isotope ratios

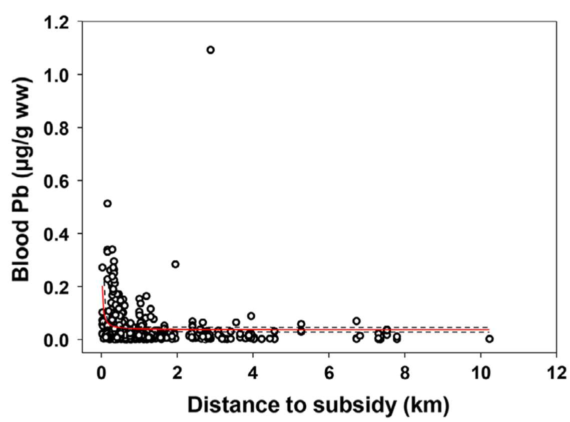

Fig. 2. Relationship between whole-blood lead (Pb) concentrations (μg/g ww) in nestling Golden Eagles (*Aquila chrysaetos*; $n = 397$) and the distance from nest to nearest subsidy field (km) in the western United States (California, Idaho, Oregon, Wyoming). Dashed lines indicate the 95% confidence interval.

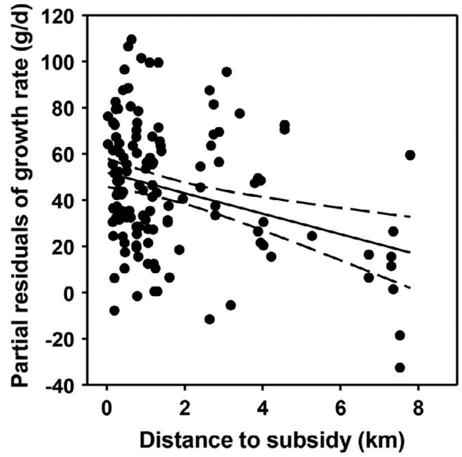

Fig. 3. Relationship between partial residuals of growth rate (g/d) and the distance to nearest subsidy (km) in nestling Golden Eagles (*Aquila chrysaetos*; $n = 131$) in the western United States (California, Idaho, Oregon, Wyoming). Dashed lines indicate the 95% confidence interval. Partial residuals are measured from a model including region.

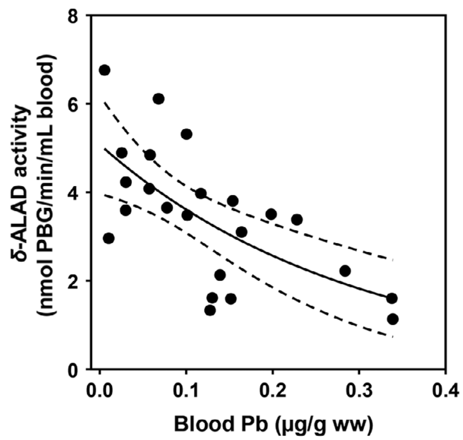

Fig. 4. Relationship between delta-aminolevulinic acid dehydratase (δ-ALAD) activity (nmol PBG/min/mL blood) and whole-blood lead (Pb) concentration (µg/g ww) in nestling Golden Eagles (*Aquila chrysaetos*; $n = 23$) in the western United States (California, Idaho, Oregon, Wyoming). Delta-aminolevulinic acid dehydratase activity declined 68% across the observed range of Pb concentrations (0.01–0.34 µg/g). Dashed lines indicate the 95% confidence interval.

were consistent with that of soil ($^{206}Pb/^{207}Pb$ = 1.199 ± 0.003; Reimann et al. 2011), 7% were consistent with background Pb ($^{206}Pb/^{207}Pb$ = 1.175 ± 0.003, e.g., historic atmospheric Pb gasoline emissions; Flegal et al. 2010), and the remaining 17% had isotopic signatures indicating a mixture of sources (Fig. 5). Stable isotope ratios in the majority of blood samples with elevated Pb concentrations above 10 µg/g ww (89%) overlapped with Pb-based ammunition, whereas stable isotope ratios from 67% of samples below 0.02 µg/g ww overlapped with those of historic atmospheric Pb gasoline emissions or soil Pb (Fig. 5).

Discussion

We provide among the most comprehensive assessments to date of Pb exposure in nestling

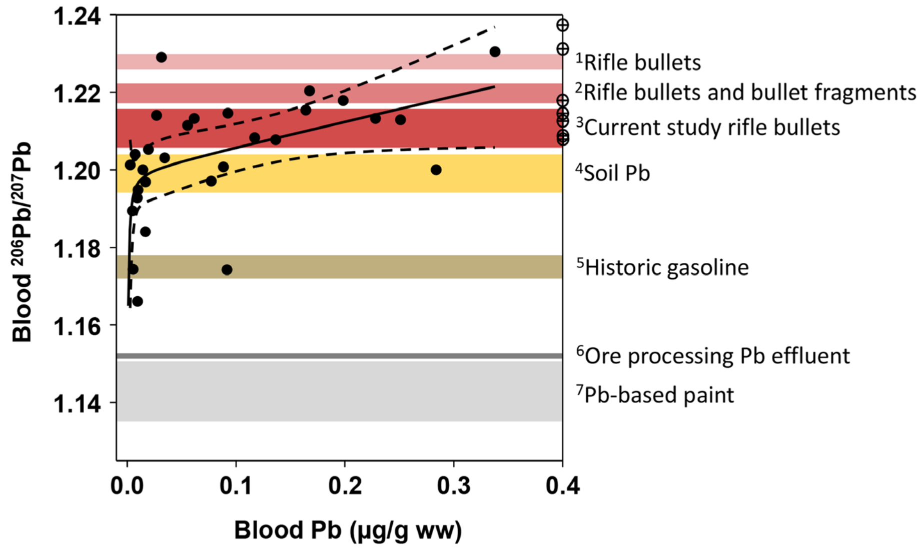

Fig. 5. Relationship between whole-blood lead (Pb) stable isotope ratios ($^{206}Pb/^{207}Pb$) and whole-blood Pb concentrations (μg/g ww) in nestling Golden Eagles (*Aquila chrysaetos; n* = 29) in the western United States (California, Idaho, Oregon, Wyoming) represented by black dots. The shaded regions represent the mean ± standard error for $^{206}Pb/^{207}Pb$ isotope ratio for Pb-based ammunition ([1]Tsuji et al. 2008, [2]Finkelstein et al. 2012, [3]Current study), background soil measured in California ([4]Reimann et al. 2011), lichen samples from northern California reflecting historic atmospheric Pb gasoline emissions ([5]Flegal et al. 2010), regional ore processing Pb effluent ([6]Shiel et al. 2010), and Pb-based paint ([7]Jaeger et al. 1998/Finkelstein et al. 2012). The crosshair symbols on the right *y*-axis represent the $^{206}Pb/^{207}Pb$ isotope ratios of Pb-based bullets sampled in the current study. Dashed lines indicate the 95% confidence interval for the relationship between blood Pb concentrations and $^{206}Pb/^{207}Pb$ isotope ratios.

Golden Eagles in North America, detailing potential drivers and sources of exposure, physiological effects, and hazards associated with Pb that are faced by nestlings. Golden Eagle nestlings experienced wide-ranging Pb exposure, with Pb levels spanning three orders of magnitude. Although most nestling Pb concentrations were relatively low (<0.03 μg/g), 26% exceeded concentrations that would impair physiological condition in Pb-sensitive species (Martínez-López et al. 2004, Finkelstein et al. 2012, Epsín et al. 2015). As such, we observed a 67% reduction in δ-ALAD activity across the range of nestling Pb concentrations. We also found strong support that habitat type was an important predictor of nestling Pb exposure. Specifically, nestlings reared near hay fields had higher Pb concentrations than those more distant from hay fields (suggesting an ecological trap dependent on proximity to shooting fields), and concentrations declined exponentially with increasing distance. Importantly, despite having higher Pb concentrations near hayfields, growth rates were higher in nestlings located closer to subsidy fields than those further away.

The proximity of nests to potential subsidies had a strong effect on Golden Eagle nestling Pb exposure; in fact, 98% of the top 10th percentile concentrations occurred within 2 km of subsidy fields, whereas 39% of nestlings further than 2 km from the nearest subsidy fields had Pb concentrations in the lower 25th percentile. Although consumption of Pb-based bullet fragments left in big game offal is commonly associated with Pb exposure in scavenging birds such as Golden Eagles (Craighead and Bedrosian 2008, Legagneux et al. 2014, Langner et al. 2015), our research occurred during the spring and early summer—outside the legal big game hunting season. Recreational and pest control shooting of ground squirrels and prairie dogs is common during this period and can result in over 100 shot animals per day per shooter left in fields (Pauli and Buskirk 2007, Herring et al. 2016). Moreover, most of the bullet fragments retained in shot ground squirrel and prairie dogs carcasses are comprised of Pb; 7–35% of the carcasses contain a potential lethal dose of Pb to raptors in a single meal (Knopper et al. 2006, Pauli and Buskirk 2007, Herring et al. 2016) assuming nestlings consumed the entire carcass and digested all the Pb fragments. Further, shooting activity can serve as a cue for attracting raptors and other avian scavengers to a site (Harmata and Restani 1995, White 2005). Although not all regions we sampled had extensive ground squirrel shooting in hay fields, recreational shooters often select natural areas near agriculture fields likely because of access (Pauli et al. 2019) and other forms of recreational shooting of agricultural pests often occur (e.g., coyotes; Stauber et al. 2010, Watson and Davies 2015).

Prey availability (prey density and vulnerability to capture) ultimately defines when and where birds forage (Boutin 1990, Gawlik 2002). However, in our study we found little support for core subsidy area, which served as a proxy for potential abundance or density of agricultural-associated prey but strong support for the distance to subsidy. This suggests that Pb exposure in Golden Eagles was driven more by the simple availability of carcasses shot with Pb-based ammunition than the actual abundance or potential density of prey at subsidy fields. In other words, adult Golden Eagles were attracted to shooting sites likely because there were shot carcasses there, not necessarily because of how much prey might have been at that subsidy field. This is an important finding because it demonstrates just how important shooting fields may be during the breeding season, if they occur within the foraging range of Golden Eagles.

Although closer proximity to potential subsidies may increase Pb exposure in nestling Golden Eagles, the potential impairment from Pb exposure may be at least partially offset by the energetic benefits of increased food availability, as evidenced by higher nestling growth rates for those birds closest to potential subsidies. As a corollary, subsidies may benefit Golden Eagles in some ways, they may also create an ecological trap where adult Golden Eagles are attracted to abundant shot ground squirrels but have no behavior cue to alert them to the risk of Pb exposure from the subsidy which causes physiological impairment in nestlings (Battin 2004, Fritsch et al. 2019). However, without evidence of a true effect on population growth rates we cannot be certain that ground squirrel shooting or other forms of pest management on hay fields truly acts as an ecological trap for breeding golden eagles, or raptors in general. One important caveat to these findings is that because of the strong relationship between distance to subsidies and Pb concentrations, we could not determine if Pb exposure influenced growth rates. However, the procedure we used to determine which variable (distance to subsidy or Pb concentration) to include in the final growth models did suggest that the relationship between nestling growth rates and distance to subsidy had more support than that between growth rates and Pb concentrations. This should not be interpreted as Pb does not influence Golden Eagle nestling growth rates; rather that for the range of Pb in nestlings the benefit of subsidies on nestling growth exceeded any impairment caused by Pb.

Understanding how prey availability differs with respect to habitat types within nest site core foraging areas would help improve our understanding of when and where Golden Eagle nestlings may be exposed to Pb and could be critical when considering potential management scenarios to mitigate Pb exposure. The degree to which proximity to hay fields results in increased prey availability and increased access to shot subsidies likely varies among landscapes, influenced by (1) the juxtaposition of hay fields and Golden Eagle nest site habitat, (2) presence of prey species that are positively associated with hay cultivation, and (3) recreational or pest control shooting that is similarly associated with hay cultivation. For example, Belding's ground squirrels (*Urocitellus beldingi*) attain very high densities within sprinkler-irrigated alfalfa fields, causing substantial economic losses that require pest control measures such as poisoning and shooting (Whisson et al. 1999), and attracting high levels of recreational shooting (Herring et al. 2016). This situation represents a spatially identifiable, focused source of Pb exposure within this squirrel's geographic range (Oregon and California study areas; Herring et al. 2016) that is more amenable to remediation than more dispersed hunting, recreational, and pest shooting (Stauber et al. 2010, Pauli et al. 2019).

The suppression of δ-ALAD activity, which is a precursor to heme synthesis and a sensitive indicator of physiological response to Pb exposure, is a common effect of Pb exposure in raptors (see Hoffman et al. 1985, Redig et al. 1991, Henny et al. 1994). However, only limited information is available regarding how the effects of low-level Pb exposure influences δ-ALAD (see Martínez-López et al. 2004, Finkelstein et al. 2012, Epsín et al. 2015). We found suppressed δ-ALAD activity (8% below reference) at blood concentrations as low as 0.03 μg/g. Moreover, the rate of suppression we measured indicates that Golden Eagle nestlings with whole-blood Pb concentrations of 0.10 μg/g ww would have 28% lower δ-ALAD activity relative to background Pb exposure. Similarly, δ-ALAD activity would be reduced by 49% and 63% when blood Pb concentrations reach 0.20 and 0.30 μg/g, respectively. The inhibition of δ-ALAD activity can result in reduction of blood hemoglobin concentrations and anemia (Ferreyra et al. 2015), as well as morphological changes to red blood cells that result in hemolysis (Pattee and Pain 2003, Mateo et al. 2003, Mitchell and Johns 2008). Further, these negative physiological effects can result in the generation of reactive oxygen species that can enhance lipid peroxidation and cause DNA damage (Gurer and Ercal 2000).

Effects of Pb exposure can manifest in many additional ways beyond reduction in δ-ALAD activity. It is unclear what effect current levels of Pb exposure and associated physiological effects might have on productivity or nestling survival, but Pb concentrations were generally not high enough to result in outright mortality (Franson and Pain 2011). Regardless, the first year of a bird's life is generally associated with the highest probability of mortality given the need for rapid growth and neurological development in order to successfully fledge and learn to survive on their own (Lack 1954, Newton 1979, McIntyre et al. 2006), thus any exposure to Pb in nestling Golden Eagles may be an additional stressor and exacerbate these difficulties. Moreover, permanent neurological impairment from experimental Pb exposure in developing rat and mouse models has been shown to manifest in lifelong deficits in behavior (Kuhlmann et al. 2009), learning (Sawyer and Strupp 1996, Li et al. 2014), and memory effects (Li et al. 2014) as adults. Similar long-term research in developing birds is lacking, but behavioral and learning impacts have been documented in response to experimental Pb dosing of common tern (*Sterna hirundo*) nestlings (Burger and Gochfeld 1985, Gochfeld and Burger 1988). Additionally, across the Golden Eagle nestlings we sampled, 34% of all nestlings had blood Pb concentrations that have been shown to impair flight in adult eagles (Ecke 2017), assuming whole-blood Pb concentrations were similar when nestlings fledged. On average, nestlings remained in the nest for an additional 2–3 weeks after blood sampling and would continue to be at risk of localized Pb exposure even after fledging.

Mitigating Pb exposure in nestling Golden Eagles requires an understanding of potential Pb sources. Pb stable isotopes provide a unique tool for differentiating potential sources of Pb, and have been employed effectively for this purpose in wildlife toxicology studies (see Finkelstein et al. 2012, Legagneux et al. 2014, Sriram et al. 2018). Consistent with other research on Pb exposure in avian scavengers, we observed a positive relationship between ^{206}Pb/^{207}Pb isotope ratios and whole-blood Pb concentrations (Church et al. 2006, Finkelstein et al. 2012, Legagneux et al. 2014). Overall, 45% of all Golden Eagle nestling samples were consistent with Pb-based ammunition as being the source of exposure, and above blood concentrations of 0.10 μg/g ww, that proportion increased to 89% of the nestlings. In contrast, ^{206}Pb/^{207}Pb isotope ratios were more variable in birds with blood Pb concentrations below 0.10 μg/g ww. Lead exposure below 0.10 μg/g ww more likely represents background environmental sources of Pb, such as historic atmospheric Pb gasoline emissions and soil exposure (Flegal et al. 2010, Reimann et al. 2011), or mixing of ammunition Pb isotope signatures with background sources.

Agricultural subsidies can benefit breeding raptors when they enhance food availability (Cortés-Avizanda et al. 2012, Oro et al. 2013), or improve the predictability of obtaining food (Bartumeus et al. 2010, Cortés-Avizanda et al. 2012). This can decrease energetic costs of foraging and improve physiological condition and breeding performance (Robb et al. 2008, Oro et al. 2013).

Potential subsidies, such as ground squirrels, available to Golden Eagle nestlings in close proximity to agricultural fields were associated with higher growth rates than in nestlings more distant from fields, where shooting occurred. However, there is concomitant Pb exposure and associated decreased δ-ALAD activity which can have cascading effects on their health (Mitchell and Johns 2008, Ferreyra et al. 2015, Gurer and Ercal 2000). Understanding how these observed effects (positive and negative) influence Golden Eagles as they transition into fledglings and subadults may help put these results into a broader demographic perspective and provide conservation managers with critical information from for evaluation of proposed measures taken to reduce Pb exposure across the landscape associated with recreational shooting and agricultural pest management.

Acknowledgments

This research was funded by the Western Golden Eagle Team, U.S. Fish and Wildlife Service, and U.S. Geological Survey (Environmental Health Mission Area—Contaminant Biology Program and Ecosystems Mission Area). In Idaho, additional funding came from the Bureau of Land Management (L14AC00342), the National Science Foundation Division of Biological Infrastructure (1263167), and the Boise State University Raptor Research Center, and M.N. Kochert and K. Steenhof contributed technical and logistical assistance. In Wyoming, additional funding came from Wyoming Wildlife Foundation, Bureau of Land Management, Rocky Mountain Power, and Nancy-Carroll Draper Foundation. We thank J. Anderson, S. Anderson, E. Arnold, L. Broduer, L. Brown, N. Ciaravella, R. Claremont, J. Cordova, S. Crane, C. Davis, M. Dirkson, T. Ely, E. Forsman, S. Ginger, B. Glenn, J. Goodell, G. Grant, C. Hallet, J. Harrison, M. Hedrick, M. Henderson, M. Hill, N. Horton, F. Isaacs, M. Kreb, R. Jones, K. Lahusen, A. Leal, B. Lawrence-Smith, P. Lickwar, M. Lipsitz, B. Mandish, A. Maurer, E. Materna, R. Migala, J. Nelson, S. Norton, J. Pierce, B. Price, C. Rumrill, D. Shepherdson, M. Stuber, F. Toland, B. Tueler, A. Ungerer, D. Varland, R. Vetter, A. Wilhelm, E. Williamson, E. Volk, and S. Wray for field and laboratory work. M. Ricca and two anonymous reviewers provided valuable reviews of earlier drafts of this manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The findings and conclusions presented in this paper are those of the authors and do not necessarily reflect the views of the U.S. Fish and Wildlife Service; however, this product paper has been peer-reviewed and approved for publication consistent with USGS Fundamental Science Practices (http://pubs.usgs.gov/circ/1367/). The dataset analyzed during the current study is available from the corresponding author on reasonable request.

Literature Cited

Almasi, B., P. Beziers, A. Roulin, and L. Jenni. 2015. Agricultural land use and human presence around breeding sites increase stress-hormone levels and decrease body mass in barn owl nestlings. Oecologia 179:89–101.

Andersen, K. A. 1996. Micro-digestion and ICP-AES analysis for the determination of macro and micro elements in plant tissues. Atomic Spectroscopy 17:30–33.

Arnold, T. W. 2010. Uninformative parameters and model selection using Akaike's Information criterion. Journal of Wildlife Management 74:1175–1178.

Arroyo, B., J. T. Garccia, and V. Betagnolle. 2002. Conservation of the Montagu's harrier (*Circus pygargus*) in agricultural areas. Animal Conservation 5:283–290.

Bartumeus, F., L. Giuggioli, M. Louzao, V. Bretagnolle, D. Oro, and S. A. Levin. 2010. Fishery discards impact on seabird movement patterns at regional scales. Current Biology 20:215–222.

Battin, J. 2004. When good animals love bad habitats: ecological traps and the conservation of animal populations. Conservation Biology 18:1482–1491.

Bedrosian, G., J. W. Watson, K. Steenhof, M. N. Kochert, C. Preston, B. Woodbridge, G. E. Williams, K. R. Keller, and R. H. Crandall. 2017. Spatial and temporal patterns in golden eagle diets in the western United States, with implications for conservation planning. Journal of Raptor Research 51:347–367.

Berlin, A., and K. H. Schaller. 1974. European standardized method for the determination of delta-aminolevulinic acid dehydratase activity in blood. Journal of Clinical Chemistry and Biochemistry 8:389–390.

Boutin, S. 1990. Food supplementation experiments with terrestrial vertebrates: patterns, problems, and the future. Canadian Journal of Zoology 68:203–220.

Burger, J., and M. Gochfeld. 1985. Early postnatal lead exposure: behavioral effects in Common Tern chicks (*Sterna hirundo*). Journal of Toxicology and Environmental Health 16:869–886.

Burnham, K. P., and D. R. Anderson. 2002. Model selection and inference: a practical information-theoretic approach. Springer-Verlag, New York, New York, USA.

Cardador, L., M. Carrete, and S. Mańosa. 2011. Can intensive agricultural landscapes favour some raptor species? The marsh harrier in north-eastern Spain. Animal Conservation 14:382–390.

Church, M. E., R. Gwiazda, R. W. Riseborough, C. P. Chamberlain, S. Farry, W. Heinrich, B. A. Rideout, and D. R. Smith. 2006. Ammunition is the principal source of lead accumulated by California Condors re-introduced to the wild. Environmental Science and Technology 40:6143–6150.

Collopy, M. W. 1986. Food consumption and growth energetics of nestling golden eagles. Wilson Bulletin 98:445–458.

Cortés-Avizanda, A., R. Jovani, M. Carrete, and J. A. Donázar. 2012. Resource unpredictability promotes species diversity and coexistence in an avian scavenger guild: a field experiment. Ecology 93:2570–2579.

Costantini, D., G. Dell'Omo, I. La Fata, and S. Casagrande. 2014. Reproductive performance of Eurasian kestrel *Falco tinnunculus* in an agricultural landscape with a mosaic of land uses. Ibis 156:768–776.

Craig, T. H., and E. H. Craig. 1984. Results of a helicopter survey of cliff nesting raptors in a deep canyon in southern Idaho. Raptor Research 18:20–25.

Craighead, D., and B. Bedrosian. 2008. Blood lead levels of common ravens with access to big-game offal. Journal of Wildlife Management 72:240–245.

Driscoll, D. E. 2010. Protocol for Golden Eagle occupancy, reproduction, and prey population assessment. American Eagle Research Institute, Apache Junction, Arizona, USA.

Ecke, F., et al. 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. Environmental Science and Technology 51:5729–5736.

Epsín, S., E. Martínez-López, P. Jiménez, P. María-Mojica, and A. J. Garcia-Fernández. 2015. Delta-aminolevulinic acid dehydratase (δALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions. Environmental Research 137:185–198.

Ferreyra, H., P. M. Beldomenico, K. Marchese, M. Romano, A. Caselli, A. I. Correa, and M. Uhart. 2015. Lead exposure affects health indices in free-ranging ducks in Argentina. Ecotoxicology 24:735–745.

Finkelstein, M. E., D. F. Doak, D. George, J. Burnett, J. Brandt, M. Church, J. Grantham, and D. R. Smith. 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proceedings of the National Academy of Sciences USA 109:11449–11454.

Flegal, A. R., C. Gallon, S. Hibdon, Z. E. Kuspa, and L. F. Laporte. 2010. Declining-but persistent-atmospheric contamination in Central California from the resuspension of historic leaded gasoline emissions as recorded in the lace lichen (*Ramalina mesnziesii* Taylor) from 1892 to 2006. Environmental Science and Technology 44:5613–5618.

Franson, J. C., and D. Pain. 2011. Lead in birds. *In* W. N. Beyer and J. P. Meador, editors. Environmental contaminants in biota, interpreting tissue concentrations. Second edition. Taylor & Francis Group, Boca Raton, Florida, USA.

Fritsch, C., L. Jankowiak, and D. Wysocki. 2019. Exposure to Pb impairs breeding success and is associated with longer lifespan in urban European blackbirds. Scientific Reports 9:489.

Garbett, R., G. Maude, P. Hancock, D. Kenny, R. Reading, and A. Amar. 2018. Association between hunting and elevated blood lead levels in the critically endangered African white-backed vulture *Gyps africanus*. Science of the Total Environment 639:1654–1665.

Gawlik, D. E. 2002. The effects of prey availability on the numeric response of wading birds. Ecological Monographs 72:329–346.

Gil-Sánchez, J. M., S. Molleda, J. A. Sánchez-Zapata, J. Bautista, I. Navas, R. Godinho, A. J. García-Fernández, and M. Moleón. 2018. From sport hunting to breeding success: patterns of lead ammunition ingestion and its effects on an endangered raptor. Science of the Total Environment 613–614:483–491.

Gochfeld, M., and J. Burger. 1988. Effects of lead on growth and feeding behavior of young common terns (*Sterna hirundo*). Archives of Environmental Contamination and Toxicology 17:513–517.

Gurer, H., and N. Ercal. 2000. Can antioxidants be beneficial in the treatment of lead poisoning? Free Radical Biology and Medicine 29:927–945.

Haig, S. M., J. D'Elia, C. E. Eagles-Smith, J. M. Fair, J. Gervais, G. Herring, J. W. Rivers, and J. H. Schulz. 2014. The persistent problem of lead poisoning in birds from ammunition and fishing tackle. Condor 116:408–428.

Haines-Young, R. 2009. Land use and biodiversity relationships. Land Use Policy 26(Supplement):S178–S186.

Hale, R., and S. E. Swearer. 2016. Ecological traps: current evidence and future directions. Proceedings of the Royal Society B 283:20152647.

Harmata, A. R., and M. Restani. 1995. Environmental contaminants and cholinesterase in blood of vernal

migrant bald and golden eagles in Montana. Intermountain Journal of Science 1:1–15.
Helsel, D. R. 2006. Fabricating data: how substituting values for nondetects can ruin results, and what can be done about it. Chemosphere 65:2434–2439.
Henny, C. J., L. J. Blus, D. J. Hoffman, and R. A. Grove. 1994. Lead in hawks, falcons and owls downstream from a mining site on the Coeur d'Alene River, Idaho. Environmental Monitoring and Assessment 29:267–288.
Herring, G., C. Eagles-Smith, J. Ackerman, D. Gawlik, and J. Beerens. 2013. Landscape factors and hydrology influence mercury concentrations in wading birds breeding the Florida Everglades, USA. Science of the Total Environment 460:637–646.
Herring, G., C. A. Eagles-Smith, and J. A. Buck. 2017. Characterizing golden eagle risk to lead and anticoagulant rodenticide exposure. Journal of Raptor Research 51:273–292.
Herring, G., C. A. Eagles-Smith, and M. T. Wagner. 2016. Ground squirrel shooting and potential lead exposure in breeding avian scavengers. PLOS ONE 11:e0167926.
Hoechlin, D. R. 1976. Development of golden eagles in southern California. Western Birds 7:137–152.
Hoekstra, J. M., T. M. Boucher, T. H. Ricketts, and C. Roberts. 2004. Confronting a biome crisis; global disparities of habitat loss and protection. Ecology Letters 8:23–29.
Hoffman, D. J., J. C. Franson, O. H. Pattee, C. M. Bunck, and A. Anderson. 1985. Survival, growth and accumulation of ingested lead in nestling American kestrels (*Falco sparverius*). Archives of Environmental Contamination and Toxicology 14:89–94.
Ishii, C., et al. 2017. Lead exposure in raptors from Japan and source identification using Pb stable isotope ratios. Chemosphere 186:367–373.
Jaeger, R. J., A. L. Weiss, and W. I. Manton. 1998. Isotopic ratio analysis in residential lead-based paint and associated surficial dust. Clinical Toxicology 36:691–703.
Jenni, L., M. M. Madry, T. Kraemer, J. Kupper, H. Naegeli, H. Jenny, and D. Jenny. 2015. The frequency distribution of lead concentrations in feathers, bone, kidney and liver of golden eagles *Aquila crysaetos* insights into the modes of uptake. Journal of Ornithology 156:1095–1103.
Kenntner, N., Y. Crettenand, H. J. Fünfstück, M. Janovsky, and F. Tataruch. 2007. Lead poisoning and heavy metal exposure of golden eagles (*Aquila chrysaetos*) from the European Alps. Journal of Ornithology 148:173–177.
Knopper, L. D., P. Mineau, A. M. Scheuhammer, D. E. Bond, and D. T. McKinnon. 2006. Carcasses of shot Richardson's ground squirrels may pose lead hazards to scavenging hawks. Journal of Wildlife Management 70:295–299.
Komárek, M., V. Ettler, V. Chrastnń, and M. Mihajeviĉ. 2008. Lead isotopes in environmental sciences: a review. Environmental International 34:562–577.
Kuhlmann, K. C., J. L. McGlothan, and T. R. Guilarte. 2009. Developmental lead exposure causes spatial learning deficits in adult rats. Neuroscience Letters 233:101–104.
Lack, D. 1954. The natural regulation of animal numbers. Oxford University Press, Oxford, UK.
Langner, H. W., R. Domenech, V. Slabe, and S. P. Sullivan. 2015. Lead and mercury in fall migrant golden eagles from western North America. Archives of Environmental Contamination and Toxicology 69:54–61.
Legagneux, P., P. Suffice, J. S. Messier, F. Lelievre, J. A. Tremblay, and C. Maisonneuve. 2014. High risk of lead contamination for scavengers in an area with high moose hunting success. PLOS ONE 9: e111546.
Li, N., F. Lui, L. Song, P. Zhang, M. Qiao, Q. Zhao, and W. Li. 2014. The effects of early life Pb exposure on the expression of IL1-β, TNF-α and Aβ in cerebral cortex of mouse pups. Journal of Trace Elements in Medicine and Biology 28:100–104.
Martínez-López, E., J. E. Martínez, P. María-Mojica, J. Peñalver, M. Pulido, J. F. Calvo, and A. J. García-Fernández. 2004. Lead in feathers and δ-aminolevulinic acid dehydratase activity in three raptor species from an unpolluted Mediterranean forest (Southeastern Spain). Archives of Environmental Contamination and Toxicology 47:270–275.
Marzluff, J. M., S. T. Knick, M. S. Vekasy, L. S. Schueck, and T. J. Zarillo. 1997. Spatial use and habitat selection of golden eagles in southwestern Idaho. Auk 114:673–687.
Mateo, R., W. N. Beyer, J. Spann, D. Hoffman, and A. Ramis. 2003. Relationship between oxidative stress, pathology, and behavioral signs of lead poisoning in Mallards. Journal of Toxicology and Environmental Health A Current Issues 66:1371–1389.
McIntyre, C. L., M. W. Collopy, and M. S. Lindberg. 2006. Survival probability and mortality of migratory juvenile golden eagles from interior Alaska. Journal of Wildlife Management 70:717–722.
McTee, M., B. Hiller, and P. Ramsey. 2019. Free lunch, may contain lead: scavenging shot small mammals. Journal of Wildlife Management 83:1466–1473.
McTee, M., M. Young, A. Umansky, and P. Ramsey. 2017. Better bullets to shoot small mammals

without poisoning scavengers. Wildlife Society Bulletin 41:736–742.

Menkens Jr., G. E., and S. H. Anderson. 1987. Nest site characteristics of a predominantly tree-nesting population of golden eagles. Journal of Field Ornithology 58:22–25.

Mitchell, E. B., and J. Johns. 2008. Avian hematology and related disorders. Veterinary Clinics of Exotic Animals 11:501–522.

Monsarrat, S., S. Benhamou, F. Sarrazin, C. Bessa-Gomes, W. Bouten, and O. Duriez. 2013. How predictability of feeding patches affects home range size and foraging habitat selection in avian social scavengers. PLOS ONE 8:e53077.

Murgatroyd, M., L. G. Underhill, L. Rodrigues, and A. Amar. 2016. The influence of agricultural transformation on the breeding performance of a top predator: Verreaux's eagles in contrasting land use areas. Ornithological Applications 118:238–252.

Naidoo, V., K. Wolter, and C. J. Botha. 2018. Lead ingestion as a potential contributing factor to the decline in vulture populations in southern Africa. Environmental Research 152:150–156.

Newton, I. 1979. Population ecology of raptors. T&A.D. Poyser, Berkhamsted, UK.

Oro, D., M. Genovart, G. Tavechia, M. S. Fowler, and A. Martínez-Abráin. 2013. Ecological and evolutionary implications of food subsidies from humans. Ecology Letters 16:1501–1514.

Panek, M., and J. Hušek. 2014. The effect of oilseed rape occurrence on main prey abundance and breeding success of the common buzzard *Buteo buteo*. Bird Study 61:457–464.

Pattee, O. H., P. H. Bloom, J. M. Scott, and M. R. Smith. 1990. Lead hazards within the range of the California condor. Condor 92:931–937.

Pattee, O. H., and D. J. Pain. 2003. Chapter 15: Lead in the Environment. *In* D. J. Hoffman, B. A. Rattner, G. A. Burton, and J. Cairns Jr., editors. Handbook of ecotoxicology. Second edition. CRC Press, Boca Raton, Florida, USA.

Pauli, J. N., and S. W. Buskirk. 2007. Recreational shooting of prairie dogs: a portal for lead entering wildlife food chains. Journal of Wildlife Management 71:103–108.

Pauli, B. P., E. R. Sun, Z. K. Tinkle, J. S. Forbey, K. E. Demps, and J. A. Heath. 2019. Human habitat selection: using tools from wildlife ecology to predict recreation in natural landscapes. Natural Areas Journal 39:500–507.

Preston, C. R., R. E. Jones, and N. S. Horton. 2017. Golden eagle diet breadth and reproduction in relation to fluctuations in primary prey abundance in Wyoming's Bighorn Basin. Journal of Raptor Research 51:334–346.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Redig, P. T., E. M. Lawler, S. Schwartz, J. L. Dunnette, B. Stephenson, and G. E. Duke. 1991. Effects of chronic exposure to sub-lethal concentrations of lead acetate on heme synthesis and immune function in red-tailed hawks. Archives of Environmental Contamination and Toxicology 21:72–77.

Reimann, C., D. B. Smith, L. G. Woodruff, and B. Flem. 2011. Pb-concentrations and Pb-isotope rations in soils collected along an east-west transect across the United States. Applied Geochemistry 26:1623–1631.

Robb, G. N., R. A. McDonald, D. E. Chamberlain, and S. Bearhop. 2008. Food for thought: supplementary feeding as a driver of ecological change in avian populations. Frontiers in Ecology and Environment 6:476–484.

Sawyer, R. L., and B. J. Strupp. 1996. Enduring alterations in response patterns in rats exposed to lead (Pb) during early development: II. Three-choice olfactory serial reversal task and an extra-dimensional shift (EDS). Neurotoxicology Teratology 18:336.

Seoraj-Pillai, N., and N. Pillay. 2017. A meta-analysis of human-wildlife conflict: South African and global perspectives. Sustainability 9:34.

Sergio, F., I. Newton, L. Marchesi, and P. Pedrini. 2006. Ecologically justified charisma: Preservation of top predators delivers biodiversity conservation. Journal of Applied Ecology 43:1049–1055.

Shiel, A. E., D. Weis, and K. J. Orians. 2010. Evaluation of zinc, cadmium and lead isotope fractionation during smelting and refining. Science of the Total Environment 408:2357–2368.

Squadrone, S., R. Orusa, S. Robetto, M. Mantia, M. Rizi, G. Colombero, E. Parolvel, and M. C. Abete. 2018. Cute lead poisoning in a Golden Eagle from Italian Alps: an evaluation to metals exposure through blood and feather analysis. Toxicology Letters 295:S251.

Sriram, A., W. Roe, M. Booth, and B. Gartrell. 2018. Lead exposure in an urban, free-ranging parrot; investigating prevalence, effect and source attribution using stable isotope analysis. Science of the Total Environment 634:109–115.

Stauber, E., N. Finch, P. A. Talcott, and J. M. Gay. 2010. Lead poisoning of bald (*Haliaeetus leucocephalus*) and golden (*Aquila chrysaetos*) eagles in the U.S. inland Pacific Northwest region-an 18-year retrospective study: 1991–2008. Journal of Avian Medicine and Surgery 24:279–287.

Stephens, R. M., A. S. Johnson, R. E. Plumb, K. Dickerson, M. C. McKinstry, and S. H. Anderson. 2008. Risk assessment of lead poisoning in raptors caused by recreational shooting of prairie dogs. Intermountain Journal of Science 13:116–123.

Terraube, J., and V. Bretagnolle. 2018. Top-down limitation of mesopredators by avian top predators: a call for research on cascading effects at the community and ecosystem scale. Ibis 160:693–702.

Tsuji, L. J. S., B. C. Wainman, I. D. Martin, C. Sutherland, J. Weber, P. Dumas, and E. Nieboer. 2008. The identification of lead ammunition as a source of lead exposure in First Nations: the use of lead isotope ratios. Science of the Total Environment 393:291–298.

U.S. Department of Agriculture. 2016. https://www.nass.usda.gov/Research_and_Science/Cropland/SARS1a.php

Walters, D. M., K. M. Fritz, and R. R. Otter. 2008. The dark side of subsidies; adult stream insects export organic contaminants to riparian predators. Ecological Applications 18:1835–1841.

Watson, J. W., and R. W. Davies. 2015. Lead, mercury, and DDE in the blood of nesting golden eagles in Columbia Basin, Washington. Journal of Raptor Research 49:217–221.

Wayland, M., and T. Bollinger. 1999. Lead exposure and poisoning in bald Eagles and golden eagles in the Canadian prairie provinces. Environmental Pollution 104:341–350.

Weis, D., C. Maerschalk, J. Barling, J. De Jong, G. A. Williams, and D. Hanano. 2006. High-precision isotopic characterization of USGS reference materials by TIMS and MC-ICP-MS. Geochemistry, Geophysics, Geosystems 7:Q08006.

Whisson, D. A., S. B. Orloff, and D. L. Lancaster. 1999. Alfalfa yield loss from Belding's ground squirrels in Northern eastern California. Wildlife Society Bulletin 27:178–183.

White, C. 2005. Hunters ring dinner bell for ravens: experimental evidence of a unique foraging strategy. Ecology 86:1057–1060.

Wilmers, C. C., and W. M. Getz. 2004. Simulating the effects of wolf-elk population dynamics on resource flow to scavengers. Ecological Modelling 177:193–208.

Wolff, A., J. P. Paul, J. L. Martin, and V. Bretagnolle. 2001. The benefits of extensive agriculture to birds: the case of the little bustard. Journal of Applied Ecology 38:963–975.

Zoffoli, H. J. O., C. A. A. Varella, N. M. B. do Amaral-Sobrinho, E. Zonta, and A. Tolón-Becerra. 2013. Method of median semi-variance for the analysis of left-censored data: comparison with other techniques using environmental data. Chemosphere 93:1701–1709.