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Small-Mammal Shooting as a Conduit for Lead Exposure in Avian Scavengers

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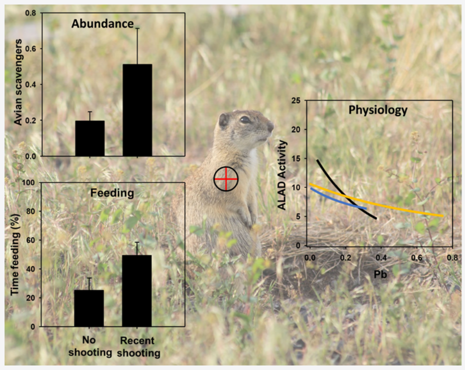

ABSTRACT: Lead (Pb) exposure is a widespread wildlife conservation threat. Although commonly associated with Pb-based ammunition from big-game hunting, small mammals (e.g., ground squirrels) shot for recreational or pest-management purposes represent a potentially important Pb vector in agricultural regions. We measured the responses of avian scavengers to pest-shooting events and examined their Pb exposure through consumption of shot mammals. There were 3.4-fold more avian scavengers at shooting fields relative to those at fields with no recent shooting, and avian scavengers spent 1.8-fold more time feeding after recent shooting events. We isotopically labeled shot ground squirrels in the field with an enriched ^{15}N isotope tracer; 6% of avian scavengers sampled within a 39 km radius reflected this tracer in their blood. However, 33% of the avian scavengers within the average foraging dispersal distance of nests (0.6–3.7 km) were labeled, demonstrating the importance of these shooting fields as a source of food for birds nesting in close proximity. Additionally, Pb concentrations in 48% of avian scavengers exceeded subclinical poisoning benchmarks for sensitive species (0.03–0.20 μg/g w/w), and those birds exhibited reduced δ-aminolevulinic acid dehydratase activity, indicating a biochemical effect of Pb. The use of shooting to manage small mammal pests is a common practice globally. Efforts that can reduce the use of Pb-based ammunition may lessen the negative physiological effects of Pb exposure on avian scavengers.

KEYWORDS: *agricultural pest management, bullet fragments, δ-aminolevulinic acid dehydratase, lead (Pb) exposure, recreational shooting, stable isotope labeling*

1. INTRODUCTION

Lead (Pb) exposure in wild birds is a global conservation issue,[1−3] affecting species across the spectrum of avian orders.[4] Although a few pathways of Pb exposure in birds (see refs 5−8) have been mitigated (e.g., Pb shot in waterfowl hunting[9,10]), Pb-based ammunition remains the most common and widespread route for avian Pb exposure.[3,4] This is particularly the case for avian scavengers, a group of birds whose foraging ecology and physiology leave them particularly susceptible to Pb exposure and toxicity.[4,8,11] The association between big-game hunting and scavenger Pb exposure is well documented in the literature, with carcass availability shown to correlate with avian Pb concentrations resulting from residual Pb fragments.[12−14] Further, Pb-based bullet fragments have been documented in the gastrointestinal tract of both dead and live birds,[15,16] and Pb stable isotope signatures from ammunition have been linked to those in avian scavengers.[8,14,17] Ultimately, this exposure has also been tied to the impairment of behavior, physiology, and survival in Pb-exposed birds.[8,17,18]

Shooting (for both pest management and recreational purposes) of small mammals such as ground squirrels and prairie dogs (hereafter, ground squirrels) may also be an important Pb exposure source in avian scavengers, particularly in agricultural regions where ground squirrel populations can be abundant and this activity is common.[17,19−21] Ground squirrel shooting as a potential source of Pb in avian scavengers differs from that of big-game hunting because of the sheer volume of shot ground squirrels (see refs 19 21, and 22) in regions where it is common. However, less is known about this pathway. Ground squirrels are major pests in these regions, reducing alfalfa (*Medicago sativa*) harvests by 17 to 66%,[23,24] and shooting is an important management tool for mitigating this damage. Land owners in agricultural regions often allow access to recreational shooters, who can kill in excess of 100 ground squirrels from a single field in a single day.[19,25] Carcasses from these shooting events are rarely retrieved or removed from the field and are thus available for consumption by scavengers.[19,20] Importantly, when shot with small-caliber (e.g., 0.177 and 0.22) Pb-based bullets, these

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carcasses contain 22.2 (±4.9 SE) Pb fragments with a mass of 7.6 mg (±3.4 SE) of Pb[19] that can then be ingested when the carcasses are consumed by avian scavengers.[19,20,22] While the aforementioned studies have demonstrated the extent to which shot ground squirrels may contain Pb fragments, they do not provide a direct understanding of how avian scavengers respond to shooting events, nor the degree to which they become exposed to Pb through carcass consumption.

To assess the potential importance of shot ground squirrels as vectors of Pb exposure and impairment to locally breeding avian scavengers, we conducted a multi-faceted study across alfalfa-growing regions in Oregon, USA, where ground squirrel shooting for pest management and recreation is common.[17,19] First, we studied the behavioral changes of avian scavengers in response to ground-squirrel-shooting activity, using avian scavenger abundance and time-activity budgets as behavioral metrics. Next, we used enriched stable isotope-labeling techniques to directly link shot carcasses from shooting events with consumption by avian scavengers and examined the distribution of exposure across the surrounding area. Finally, we assessed how the levels of Pb exposure influenced physiological responses (δ-aminolevulinic acid dehydratase; δ-ALAD) in avian scavenger blood.

2. MATERIALS AND METHODS

2.1. Study Area. We documented the avian scavenger feeding behavior and sampled birds for Pb across central and eastern Oregon, USA (Lake and Malheur Counties, Figure S1). These agricultural regions produce large amounts of irrigated alfalfa and experience extensive crop damage from Belding's ground squirrels (*Urocitellus beldingi*[23]). In addition, these regions have varying densities of ground squirrels and fluctuating ground squirrel shooting based on private land owner pest management needs and degree of public recreational shooting.[17,19] Ground squirrel shooting is unregulated, and shooting typically begins as soon as Belding's ground squirrels begin to emerge from their hibernacula (typically mid−late March). Ground squirrel shooting occurs randomly throughout this period until the ground squirrels begin hibernating, typically in August.

2.2. Description of Field Methods. *2.2.1. Behavioral Responses to Ground Squirrel Shooting.* To assess avian scavenger behavioral responses to ground-squirrel-shooting activity, we surveyed 20 fields within 48 h of the conclusion of an event (hereafter, recent shooting) between March 31 and June 16. Over 90% of avian scavengers that utilize fields after shooting arrive within 48 h of the shooting, and most ground squirrels are fully scavenged in the first 48 h.[21] We also surveyed fields more than 2 days after the last shooting event occurred on a given field (hereafter, no recent shooting). Not all field surveys had a reciprocal survey (i.e., recent shooting or no recent shooting) because the other event did not occur during the period we surveyed the field. During each survey, we continually scanned the field primarily using binoculars (10 × 42; however, a spotting scope [20−60×] was used infrequently to verify the behavior) for a 1 h period, recording the species occurrence and count of birds for each species that either landed within the boundaries of the field or were actively hunting over the field (raptors). We also kept track of perched birds and birds flying in or out of a field to avoid over- or under-counting. We standardized the total number of birds using the alfalfa fields by the field area (ha) and the proportion of the 1 h time period surveyed.

We assessed the behavioral responses of avian scavengers to ground-squirrel-shooting activity by conducting focal time-activity budgets.[26] We randomly selected a bird and observed its behavior for a 10 min period, recording its specific behavior every 30 s (six birds/survey). We defined specific behaviors as comfort (cleaning and grooming), feeding (actively capturing, handling, or ingesting prey), flight (actively hunting when recorded for longer than 30 s and constrained by the area of the alfalfa field), and perching (actively searching for prey[27]). If a bird under observation left the field before the end of the 10 min survey period, we discontinued the survey and noted the length of the truncated survey. We then selected an alternate bird and initiated a new time budget survey. All estimates of numeric response and feeding activity were adjusted to account for differences in survey time.

2.2.2. Stable Isotope Labeling. To estimate the availability of shot ground squirrels to the local avian scavenger community, we isotopically labeled shot ground squirrels with ^{15}N-enriched glycine.[28−30] We determined an appropriate level of dosing for the field study by feeding captive avian scavengers a single meal of European starling (*Sturnus vulgaris*) or mouse (*Mus musculus*) meat injected with either a 98+ at. % ^{15}N amino acid mixture (Isotec, Miamisburg, OH, USA) or 98+ at. % ^{15}N glycine (Isotec and Cambridge Isotope Lab, Tewksbury, MA, USA) in doses ranging from 0.025 to 0.322 g label kg^{-1} body mass. Birds used in the dosing experiment included bald eagles (*Haliaeetus leucocephalus* [n = 2]), common raven (*Corvus corax* [n = 1]), golden eagle (*Aquila chrysaetos* [n = 1]), and Harris hawk (*Parabuteo unicinctus* [n = 1]) housed at the High Desert Museum in Bend, OR, USA. We calculated experimental doses following the methods outlined in Pauli et al.[28] with the goal of identifying a dose that would result in a minimum of 3-fold enrichment in blood δ^{15}N of a typical scavenger for at least 30 days following consumption of a single labeled ground squirrel. We collected whole blood (0.1 mL) from the brachial vein of each captive bird for δ^{15}N analysis on days 0 (prior to dosing), 1, 3, 7, 14, and 35 post-dosing. To calculate the dose used in our field study, we assumed a median raptor mass of 1300 g and that 150 g of ground squirrel was consumed in a typical meal,[31] with the average ground squirrel weighing 228.0 ± 8.8 SE.[19] We used 9 and 5‰ as the typical natural abundance δ^{15}N of avian scavenger blood and ground squirrel muscle tissue, respectively, based on initial ^{15}N isotope analysis of wild avian scavengers (common ravens [n = 4] and golden eagles [n = 7]) and ground squirrels (n = 5) sampled in our study area during 2013 (see refs 17 19, and 32 for details). We also assumed an enrichment efficiency of 90% label incorporation based on our experiment with captive birds.

To conduct the field isotope-labeling study, we collected 829 shot Belding's ground squirrel carcasses from three shooting fields immediately after four separate shooting events over 4 separate days and stored them cooled for less than 10 h. Each carcass was injected with 5 mL of 0.2 g/mL 98+ at. % ^{15}N glycine using a 23-gauge syringe. This was equivalent to a dose of 0.15 g label kg^{-1} body mass for a 1300 g raptor based on the average daily requirement of 150 g. The label was injected throughout the carcass to ensure that birds eating only part of the carcass would still exhibit enriched isotope ratios. We then returned isotopically labeled carcasses to two fields (on 5th, 7th, 12th, and 14th May) where recent shooting had occurred, placing 472 dosed carcasses in one field and 357 in the other field. We placed carcasses out in the afternoon after ground

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squirrel shooting ceased and avian scavengers would normally have maximum access to shot carcasses. Fields used for deploying ^{15}N-labeled shot ground squirrels were adjacent to other irrigated alfalfa fields where shooting occurred.

2.2.3. Stable Isotope Sampling and Pb Exposure. We sampled avian scavengers between 24 March and 14 June throughout the study area using a combination of live capture (raptors) using bal-chatri noose traps[33,34] and lethal collections (common raven and turkey vulture only; shotgun with a non-Pb shot). To avoid resampling released individuals, we leg-banded raptors with aluminum U.S. Geological Survey bands. We collected whole blood from each bird using 20- to 25-gauge sodium-heparinized needles from either the brachial vein or by cardiac puncture (lethal collections only), and transferred blood to labeled ethylenediaminetetraacetic acid vacutainers placed immediately on ice in the field and then stored frozen at −20 °C until analysis. All field protocols were covered under state (Oregon: 021-14, 062-13, 009-14, and 064-15) and federal permits (20786, 21417, 23840, MB28361A-0, and MB834548-10) and an approved Institutional Animal Care and Use Permit (4428).

2.3. Laboratory Methods. *2.3.1. Stable Isotopes.* Stable ^{15}N isotope ratios were measured by the University of Alaska Anchorage Stable Isotope Laboratory (baseline natural abundance $\delta^{15}N$ values of wild raptors and ground squirrels) or the University of California Davis Stable Isotope Facility (captive birds and post-labeling wild birds) using continuous-flow isotope ratio mass spectrometry (Thermo Finnigan Delta Plus XP, San Jose, CA, or PDZ Europa 20-20 Sercon Ltd., Cheshire, United Kingdom; Methods S1).

2.3.2. Blood Pb Determination. We used a combination of inductively coupled plasma mass spectrometry (Thermo Scientific X-Series II CCT, Thermo Fisher Scientific, Waltham MA) and anodic stripping voltammetry (LeadCare II, Magellan Diagnosis, North Billerica, MA, USA) to determine Pb concentrations in blood (refs 35 and 36; Methods S2).

2.3.3. Physiology. To determine δ-ALAD activity, we used an adaptation of the European standard method (ref 37; Methods S3).

2.4. Statistical Methods. *2.4.1. Avian Scavenger Behavioral Responses to Ground Squirrel Shooting.* We used a linear mixed-effect model to examine the influence of shooting activity (recent vs none), avian group (raven vs raptor), and calendar date (hereafter, standard date) on the total number of avian scavengers foraging on ground squirrel fields. We taxonomically separated scavengers (ravens vs raptors) in the analysis because of differences in hunting and or scavenging behaviors that may influence time spent feeding. We excluded six data points from ring-billed gulls because they did not breed within our study area and did not represent either species group in the analysis. We included avian group × shooting activity and avian group × date interactions and removed interactions when not significant ($P > 0.05$). The influence of shooting activity on scavenger behaviors (comfort, feeding, flying, and perching) was tested using a linear mixed-effects model that included species group (raven vs raptor), date, and shooting activity (recent vs none) as factors. We analyzed the raw behavior data after determining that neither an arcsine nor logit transformation on the behavior data improved the normality of the residuals or the variance structure.[38] In both models above, we included the sampling site as a random effect to avoid confounding effects associated with sampling multiple birds at the same location.

2.4.2. Stable Isotope Labeling. To be conservative, we considered birds to be enriched (i.e., having fed on a labeled carcass) if their $\delta^{15}N$ values were above the mean plus three standard deviations (hereafter, background distribution) of the natural abundance (i.e., collected prior to the distribution of labeled ground squirrel carcasses) avian scavengers sampled from the area ($n = 18$). The estimate of the natural abundance of stable isotope ratios in birds was determined from the 11 wild birds used to establish the background ^{15}N signature in the experiment as described above, and we included an additional seven avian scavengers (six common ravens and one turkey vulture) sampled in the same year but prior to ^{15}N labeling in our study site. We plotted the relationship between $\delta^{15}N$ and the distance from the labeled fields, including $\delta^{15}N$ values from avian scavenger blood collected prior to labeling from our study sites ($n = 18$). We omitted ^{15}N data from one bird sampled 79 km away from the ^{15}N-labeled fields because the bird was sampled outside of the study area. Data from this bird are included in subsequent figures as a reference but are not included in any formal analysis.

2.4.3. Pb Exposure. We examined the influence of species and date on Pb concentrations using linear mixed-effects models. We included the sampling site as a random effect to avoid confounding effects associated with sampling multiple birds at the same location.

2.4.4. Physiology of δ-ALAD. Because δ-ALAD activity generally follows an exponential decline with increasing Pb exposure,[8,17] linear mixed-effect models are not appropriate. Therefore, we fit species-specific exponential decline models for species with sufficient sample sizes (common raven, red-tailed hawk, and Swainson's hawk). All statistical analyses were conducted using program JMP (JMP 12; SAS Institute, Cary, North Carolina, USA).

3. RESULTS

3.1. Avian Scavenger Behavioral Responses to Ground Squirrel Shooting. Twelve avian species were active in fields with no history of recent shooting activity, similar to the 11 species where recent shooting activity occurred (Table S1). There were no significant avian group × shooting activity ($F_{1,29.19} = 25$, $P = 0.62$) or avian group × date ($F_{1,32.22} = 2.20$, $P = 0.15$) interactions. Our final models indicated that there were 3.4-fold more individual avian scavengers using fields soon after recent ground-squirrel-shooting activity than during time periods not associated with shooting activity ($F_{1,31.90} = 11.09$, $P = 0.002$; Figure 1). We found no effects of date ($F_{1,29.92} = 0.22$, $P = 0.64$) or species group ($F_{1,35.00} = 1.37$, $P = 0.25$) on the number of birds in these fields. Across all feeding, flight, and perching behavioral activities, the avian group × date, avian group × shooting activity, and date × shooting activity interactions were not significant ($P > 0.15$ for all tests). However, for the comfort behavior, there was an avian group × shooting activity interaction ($F_{1,6.89} = 7.04$, $P = 0.03$), but no effect of the avian group × date ($F_{1,17.25} = 1.20$, $P = 0.29$) or date × shooting activity ($F_{1,8.34} = 2.08$, $P = 0.19$) was observed. After removing all interactions from the feeding, flight, and perching behavioral models, the avian scavenger feeding behavior was 1.8-fold higher following recent shooting activity than that when fields remained undisturbed ($F_{1,76.17} = 7.29$, $P = 0.008$; Figure 1), but it did not change with date ($F_{1,74.13} = 0.51$, $P = 0.48$) or with the species group ($F_{1,72.28} = 0.13$, $P = 0.72$). However, the flying behavior was 1.6-fold lower when there

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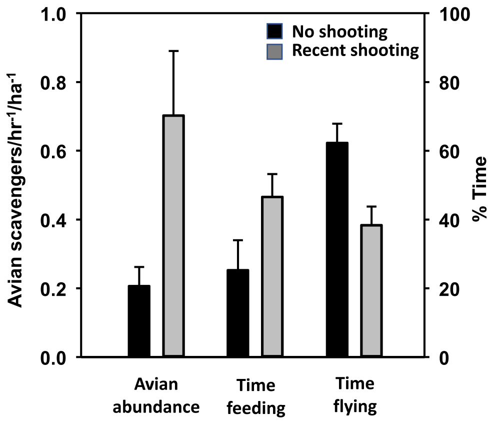

Figure 1. Avian scavenger abundance (avian scavengers/h^{-1}/ha^{-1}; left axis) and percent of time spent feeding and flying (right axis) in response to ground-squirrel-shooting activity (no shooting/recent shooting) in Oregon alfalfa-growing regions during 2014. Concentrations are model-derived least-squares means ± standard error controlling for species group, date, and shooting activity.

was recent shooting activity at fields ($F_{1,51.22}$ = 10.97, P = 0.002; Figure 1) and decreased with date (β = −0.007 ± 0.004, $F_{1,43.30}$ = 14.16, P < 0.001) but did not differ between species groups ($F_{1,90.07}$ = 0.49, P = 0.49). Raptor perching behavior was 1.2-fold higher than that of common ravens ($F_{1,96.47}$ = 4.21, P = 0.04), and the perching behavior increased with date ($F_{1,44.78}$ = 3.93, P = 0.05) but not with the shooting activity ($F_{1,63.04}$ = 0.12, P = 0.74). After running the comfort behavior model for common ravens and raptors separately, we found no effect of shooting activity ($F_{1,2.97}$ = 1.22, P = 0.35; $F_{1,1.14}$ = 0.83, P = 0.51) or date ($F_{1,3.21}$ = 0.19, P = 0.69; $F_{1,11.64}$ = 1.24, P = 0.29).

3.2. Stable Isotope Labeling. We observed a 2.7−15.8-fold increase in δ^{15}N ratios in the blood of captive avian scavengers 1 day after a single ^{15}N-enriched meal, and blood δ^{15}N remained 2.0−6.7-fold higher than natural abundance 35 days post-dosing (Figure 2).

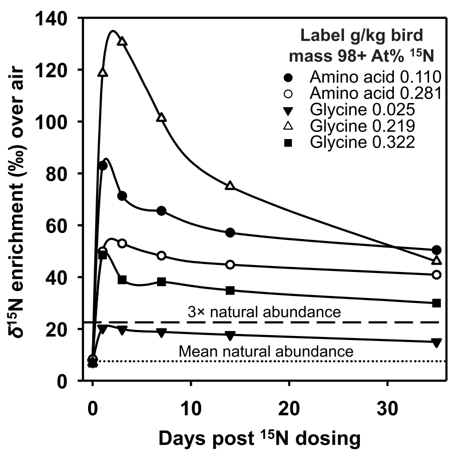

Figure 2. Experimental δ^{15}N enrichment in avian scavenger blood relative to the label dose (g kg^{-1} bird mass 98+ at. % ^{15}N) to determine optimal ground squirrel labeling for field trails. Mean natural abundance is defined as the baseline (0 days) δ^{15}N measured in each bird's blood used in the experiment. Dosing experiments were conducted using captive birds (bald eagles [n = 2], common raven [n = 1], golden eagle [n = 1], and Harris hawk [n = 1]) at the High Desert Museum, Bend, Oregon, during 2014.

We sampled 68 avian scavengers during a 30 day time period after deploying ^{15}N-enriched ground squirrel carcasses onto alfalfa fields; common raven (n = 26), red-tailed hawk (n = 19), Swainson's hawk (n = 19), and turkey vulture (n = 4). Six percent of all avian scavengers sampled after ^{15}N labeling exceeded the background distribution for background avian scavenger blood δ^{15}N (Figure 3) and were classified as labeled

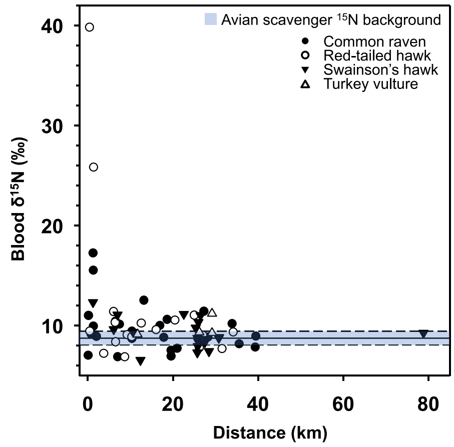

Figure 3. Relationship between the δ^{15}N (‰ vs atmospheric nitrogen) ratio of avian scavenger blood (n = 68) and the distance to the shooting field (km) where isotopically enriched carcasses were deployed. Solid line surrounded by the light blue box represents the background avian scavenger (n = 18) reference δ^{15}N isotope ratio mean ± 95% confidence interval (dashed lines).

with ^{15}N. In addition, 33% of avian scavengers sampled within the mean nesting foraging dispersal distance (0.6−3.7 km[39−43]) were labeled. The average δ^{15}N ratios of these labeled birds was 2.8-fold higher than that of background δ^{15}N ratios. The geometric mean distance between where the labeled birds were captured and the nearest labeling field was 1.0 ± 0.7 km (range = 0.4−1.3 km; Figure 3).

3.3. Pb Exposure. We measured blood Pb concentrations in 161 adult avian scavengers across five species (common raven, prairie falcon—*Falco mexicanus*, red-tailed hawk—*Buteo jamaicensis*, Swainson's hawk—*Buteo swainsoni*, and turkey vulture; Figure S1 and Table S1). Blood Pb concentrations (μg/g w/w) ranged from 0.002 to 1.102 μg/g w/w across all birds sampled, and the geometric mean (±standard error) Pb concentration was 0.053 ± 0.006 μg/g w/w (Table 1). Pb concentrations in 34% of birds were below any known impairment benchmarks; whereas 48% of avian scavengers contained Pb concentrations associated with subclinical poisoning in sensitive species (reduced δ-ALAD activity; 0.03−0.2 μg/g[8,17,44,45]). Pb concentrations in 14% of birds sampled exceeded subclinical poisoning benchmarks (0.2−0.5 μg/g[46]), 3% exceeded values associated with clinical poisoning (0.5−1.0 μg/g[46]), and 1% had concentrations deemed to cause severe clinical poisoning (>1.0 μg/g;[46] Table 1).

Whole blood Pb concentrations differed among species ($F_{4,151.00}$ = 13.38, P < 0.0001). Pb concentrations in turkey vulture and common raven were similar (Tukey's HSD: P = 0.99) and higher or marginally higher than those in red-tailed hawks (Tukey's HSD: P < 0.001 and P = 0.07, respectively; Figure 4A). Turkey vulture blood Pb concentrations were similar to those of prairie falcons (Tukey's HSD P = 0.39) and Swainson's hawks (Tukey's HSD: P = 0.42), whereas common

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Table 1. Blood Pb Concentrations in Avian Scavengers Associated with Ground-Squirrel-Shooting Fields in Oregon Alfalfa-Growing Regions during 2014[a]

species	sample size	geometric mean (μg/g w/w)	median (μg/g w/w)	range (μg/g w/w)	subclinical poisoning for sensitive species[b] (0.03−0.20 μg/g) (%)	subclinical poisoning (0.20−0.50 μg/g) (%)	clinical poisoning (0.5−1.0 μg/g) (%)	severe clinical poisoning (>1.0 μg/g) (%)
Avian Scavengers								
overall	161	0.053	0.055	0.002−1.102	48	14	3	1
common raven	68	0.106	0.083	0.005−0.751	71	21	6	
prairie falcon	6	0.029	0.028	0.004−0.318	33	7		
red-tailed hawk	41	0.023	0.021	0.003−0.318	27	9		
Swainson's hawk	42	0.040	0.032	0.002−1.102	41	9	2	2
turkey vulture	4	0.090	0.069	0.037−0.384	88	12		

[a]The percent of avian scavengers exceeding Pb exposure thresholds is based on Martínez-López et al.,[44] Franson and Pain,[46] Finkelstein et al.,[8] Espín et al.,[47] and Herring et al.[17] [b]Reduced δ-ALAD activity.

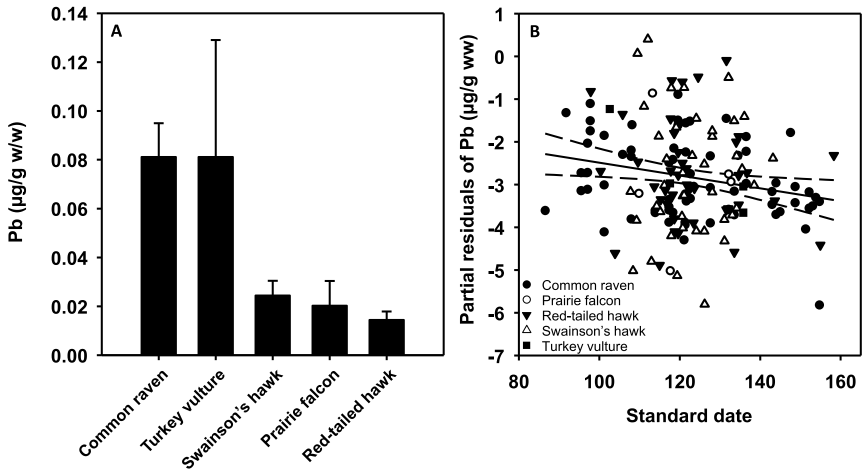

Figure 4. Lead (Pb) concentrations (μg/g wet weight) associated with factors of avian scavenger species (A) and standard date (B) sampled from Oregon alfalfa-growing regions during 2014. Concentrations are model-derived least-squares means ± standard error, controlling for spatial variation associated with the sampling site.

raven whole blood Pb concentrations were higher than those of Swainson's hawks (Tukey's HSD: $P < 0.001$) and marginally higher than those of prairie falcons (Tukey's HSD: $P = 0.05$; Figure 4A). Lastly, prairie falcon, Swainson's hawk, and red-tailed hawk whole blood Pb concentrations were all similar (all Tukey's HSD: $Ps > 0.05$; Figure 4A). Across all species, whole blood Pb concentrations declined with date ($F_{1,55.14} = 8.68$, $P = 0.005$; Figure 4B).

3.4. Physiology of δ-ALAD. There was a negative exponential relationship between blood δ-ALAD activity (nmol PBG/min/mL) and Pb concentrations (μg/g w/w) in common ravens ($P = 0.01$, $R = 0.65$), whereas the δ-ALAD activity followed a similar but nonsignificant decline in red-tailed hawks ($P = 0.14$, $R = 0.18$) and Swainson's hawk ($P = 0.49$, $R = 0.20$; Figure 5). The δ-ALAD activity was also 55% lower ($t_{91} = 3.32$, $P = 0.001$; mean = 4.6 ± 0.8 SE) in birds with Pb concentrations greater than 0.2 μg/g, relative to those with Pb concentrations below the benchmark (mean = 10.0 ± 0.5 SE).

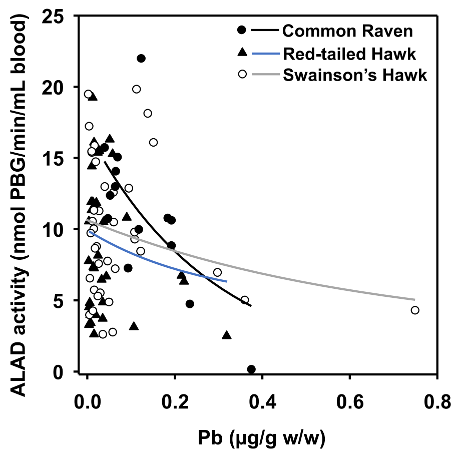

Figure 5. Relationship between the δ-ALAD activity (nmol PBG/min/mL blood) and the whole blood lead (Pb) concentration (μg/g w/w) in avian scavengers associated with ground squirrel shooting in Oregon alfalfa-growing regions during 2014.

4. DISCUSSION

Management and recreational shooting of ground squirrels play important roles in farming and ranching regions to help limit crop or field damage,[23,24] and shooting is a commonly employed technique, despite the limited evidence of effectiveness in reducing pest populations.[23,24] Our data suggest that these shooting events attract avian scavengers and can expose them to Pb through carcass consumption if Pb-based ammunition is used. We observed 3.4-fold more avian scavengers in fields with recent shooting, and they spent 1.8-fold more time actively feeding, in comparison to that in fields with no recent shooting. However, the flight time (a form of hunting[27]) was 1.6-fold lower in fields with recent shooting, suggesting that avian scavengers did not need to actively hunt as much to locate prey. Shot ground squirrels were an important prey resource for avian scavengers. Six percent of all birds and 33% of birds within the averaging foraging dispersal distance sampled after ^{15}N labeling were enriched relative to natural abundance, and all ^{15}N-labeled birds were captured less than 2 km from the fields where labeled ground squirrels were deployed. Additionally, nearly half of avian scavengers contained Pb concentrations associated with subclinical poisoning for sensitive species, and δ-ALAD activity declined in those species with a broad range of Pb concentrations.

Shooting activity is known to attract avian and mammalian scavengers.[21,48,49] Auditory cues have been confirmed to attract avian scavengers to shooting sites, conceivably explaining the large number of avian scavengers present at our survey fields either during and after shooting events.[49,50] Additionally, avian scavengers at our sites may have responded to visual cues, including other birds flying to the fields, actively scavenging within the fields, or the presence of shooting platforms as cues. The large numbers of shot ground squirrels left in the fields may have also been important in attracting new birds to these fields and having them return on subsequent days.[21,51,52]

Our ^{15}N-labeling experiment suggests that avian scavengers may not travel long distances (mean distance from ^{15}N-labeled fields = 1.0 ± 0.7 km) to feed at shooting fields. Assuming that avian scavengers were sampled close to their nesting sites, the average distance of 1.0 km is well within the range of foraging dispersal for nesting avian scavengers (0.6−3.7 km[39−43]). Considering the average foraging dispersal of nesting avian scavengers of 0.6−3.7 km, our ^{15}N-labeling experiment effectively labeled 33% (4/12) of the avian scavengers sampled within the foraging dispersal range, assuming that they were all nesting. The $\delta^{15}N$ values of field-labeled avian scavengers were considerably lower than those from our captive study, suggesting that adult avian scavengers were not consuming the entire ground squirrel carcass and likely provisioning nestlings with the remaining ^{15}N-labeled ground squirrels. Regardless, our results demonstrate the importance of these shooting fields to avian scavengers nesting within close proximity. Previous research has demonstrated that the nestling avian scavengers closest to fields where ground squirrel shooting can occur also have the highest blood Pb concentrations.[17]

Lead exposure varied by over 550-fold among the avian scavengers we sampled for this study; the differences between species likely reflect their foraging ecology and in particular the scavenging behavior.[53,54] However, given the short half-life (∼14 days) of Pb in blood,[55,56] rapid depuration may explain the large number of samples with background Pb exposure. Not surprisingly, common ravens and turkey vultures (two species that either rely heavily or exclusively on scavenging) had the highest Pb concentrations, whereas species that are more predatory, such as hawks and falcons, had lower Pb exposure. Obligate scavengers such as turkey vultures often have elevated Pb exposure (see refs 13 and 32) because of their exclusive scavenging diet, and facultative scavenger species such as common ravens are well-adapted to finding predictable food subsidies such as shot squirrels,[14,49,50,57] resulting in elevated Pb exposure.[12,32,58] In contrast, the extent to which Swainson's hawks, prairie falcons, and red-tailed hawks facultatively scavenge and subsequently are exposed to Pb in shot ground squirrels is likely driven by their proclivity for actively hunting versus facultatively scavenging,[40,59,60] and as such, they have lower Pb exposure.

Scavenging predictable pulsed resources such as shot ground squirrels may provide a valuable food source for avian scavengers while also exposing them to Pb.[17,57] However, later in the growing season, increased alfalfa height may obscure the detection of ground squirrels to both shooters and avian scavengers.[23] We observed a decline in Pb concentrations across all avian scavengers with respect to date, suggesting that avian scavengers were consuming fewer ground squirrels shot with Pb-based ammunition in the latter part of our study. This decrease in Pb concentrations in avian scavengers could be due to fewer ground squirrels being shot and available for scavenging, less scavenging of carcasses, ground squirrels estivation, or a combination thereof. Similarly, Pb concentrations were markedly lower in common ravens in Wyoming and Quebec, Canada, when fewer Pb-contaminated Rocky Mountain elk (*Cervus elaphus nelsoni*) and moose (*Alces alces*) gut piles were available (refs 12 and 14, respectively). Removal of Pb-laced ground squirrel carcasses from fields would likely result in a reduction in Pb exposure in avian scavengers.

Reduced δ-ALAD activity has been associated with a range of physiological effects[61,62] and linked to Pb exposure across multiple avian species.[5,7,8,44,47] In this study δ-ALAD activity was significantly reduced in common ravens with higher Pb concentrations and activity declined (although not significantly) in red-tailed hawks and Swainson's hawks as Pb exposure increased. Previous research has demonstrated that turkey vulture δ-ALAD activity also decreased at Pb concentrations similar to those reported in this study.[32] Although δ-ALAD activity was not significantly reduced by increasing Pb concentrations in red-tailed or Swainson's hawks, previous studies have shown that for Accipitriformes and Falconiformes, δ-ALAD activity declined more consistently at higher concentrations of Pb.[5,63,64]

5. CONSERVATION AND MANAGEMENT IMPLICATIONS

Ground squirrel shooting poses a risk to avian scavengers through secondary Pb poisoning, and that risk is largely untracked by resource managers. Because individuals are not required to have a hunting license to shoot ground squirrels on private property and there are no specified shooting seasons, the scale of this risk is relatively unknown. Species such as Swainson's hawks (species of special concern in many western states because of declining population estimates[59]) experienced some of the highest overall individual Pb exposure, which may be exacerbated by the species' propensity to nest in higher numbers in alfalfa-growing regions[39] where ground squirrel shooting is common.[19,20,22] Although we did not assess nestling Pb exposure in this study, recent research on nestling golden eagles in the western United States demonstrated that golden eagle nestlings experienced wide-ranging Pb exposure that was linked to Pb-based ammunition and associated physiological effects when located near alfalfa-growing fields.[17]

Recent studies have demonstrated that both avian and mammalian scavengers forage at ground-squirrel-shooting

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fields (ref 21, this study). Additionally, recent research has demonstrated considerable differences in the amount of Pb retained among ground squirrels shot with different calibers of Pb-based ammunition[19,20] and that non-Pb alternative ammunition may be equally as effective at killing ground squirrels.[20] Thus, the risk of Pb exposure to avian scavengers may differ depending on the type of Pb-based ammunition used and that the risk may be mitigated by non-Pb-based ammunition if it is used within the specified ballistic constraints.[65]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c01041.

> Methods for stable isotope ratio measurements, blood Pb determination, and δ-ALAD activity analysis; locations of sampling throughout the study area; incorporation of dietary ^{15}N isotope into avian scavengers; evidence of experimental $\delta^{15}N$ enrichment in birds; and avian scavengers surveyed at alfalfa fields during the 2014 growing season (PDF)

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Notes

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