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Research Article

Widespread Lead Exposure in Golden Eagles Captured in Montana

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ABSTRACT Lead poisoning threatens many species of raptors, including golden eagles (*Aquila chrysaetos*). Much of this lead likely comes from bullet fragments that remain in the carcasses of animals killed by hunters. The likelihood of lead exposure may peak during fall hunting seasons and early winter until carcasses from hunting become scarce. From 2011 to 2018 in western Montana, USA, we captured 91 golden eagles in winter, tested their blood lead levels (BLL), and outfitted a subset of birds ($n = 29$) with global positioning system [GPS] transmitters. Nearly all golden eagles (94.5%) had elevated BLL (≥10 μg/dL), and 8 of them had BLL above clinical exposure (>60 μg/dL), where they may lose coordination and experience a host of other neurological and physiological disorders. Golden eagles caught late in winter tended to have lower BLL than those caught earlier. At least 69% of the golden eagles equipped with GPS transmitters migrated northward, spending the summer throughout Alaska, USA, and northwestern Canada. Blood lead levels did not differ between migratory and nonmigratory golden eagles. Overall, elevated BLL are widespread among golden eagles throughout winter in western Montana. Promoting nonlead hunting ammunition in areas with high densities of golden eagles will reduce the birds' lead exposure. © 2020 The Authors. *The Journal of Wildlife Management* published by Wiley Periodicals LLC on behalf of The Wildlife Society.

KEY WORDS ammunition, bullets, hunting, migratory, nonlead, raptor, scavenger, transmitter.

Lead particles that remain in offal piles and hunted wildlife threaten opportunistic, scavenging birds in many regions of the world (Haig et al. 2014). Much of this lead appears to originate from lead-based rifle bullets used for hunting (Church et al. 2006, Finkelstein et al. 2012, Haig et al. 2014, Krone 2018). Lead bullets often fragment when they strike animals, so offal piles and carcasses can contain hundreds of bullet fragments that scavengers can ingest (Hunt et al. 2006, McTee et al. 2017). These small fragments of lead have more surface area relative to volume and can be dissolved more readily than a single, larger particle of equal mass, resulting in a higher uptake of lead (Barltrop and Meek 1979). Stauber et al. (2010) reported that 62% of the golden eagles admitted for rehabilitation between 1991 to 2008 in the inland Pacific Northwest had blood lead levels (BLL) ≥ 20 μg/dL, which is well above what many experts consider background concentrations (<10 μg/dL; Neumann 2009, Bedrosian et al. 2012, Langner et al. 2015). Golden eagles that have lead poisoning may experience nervous system impairment, loss of strength, and death (Pattee et al. 1981, Stauber et al. 2010, Haig et al. 2014). Considering that many golden eagles migrate (McIntyre et al. 2008, Bedrosian et al. 2018), point-sources of lead contamination, such as areas with intense hunting, may affect golden eagles that summer in different regions of western North America.

Golden eagle migration in late summer and early fall coincides with the start of big game hunting seasons. As hunting seasons proceed, the availability of lead from bullet fragments in offal piles and carcasses likely increases. On the Rocky Mountain Front in western Montana, USA, Langner et al. (2015) reported that 58% of migratory golden eagles had elevated BLL in fall. Some golden eagles had BLL high enough for the bird to be considered lethally exposed (>120 μg/dL). The mean half-life of lead in golden eagles is assumed to be about 14 days, based on work with California condors (*Gymnogyps californianus*; Fry et al. 2009). Some ingested lead can be stored in tissues and later remobilize into the blood (Rabinowitz et al. 1976), so repeated exposures may maintain or further elevate BLL in golden eagles. By December and continuing into winter, golden eagles have had the opportunity to scavenge carrion left by hunters for several consecutive months (Stauber et al. 2010, Bedrosian et al. 2012). Consequently, the highest incidences of elevated BLL may occur at the end of the hunting season and into winter. Wildlife rehabilitation centers in the United States often report an increase in the number of golden eagles they receive with elevated BLL following the hunting season (Kramer and Redig 1997, Stauber et al. 2010).

Blood lead levels may be higher for nonmigratory golden eagles than migratory ones. Golden eagles that live all year

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in the contiguous United States and more populated regions of Canada can ingest lead by eating shot prairie dogs (*Cynomys* spp.) and ground squirrels (*Urocitellus* spp.) during spring and summer (McTee et al. 2017, 2019; Herring et al. 2020). That lead may accumulate in bodily tissues and later reenter the blood, further elevating BLL after subsequent exposures. In contrast, migratory golden eagles may not consume lead during summer in areas that have less of a human presence. Nestlings in Alaska, USA, for example, have low levels of lead in their blood, if any is detected (Alaska Department of Fish and Game, unpublished data), suggesting the environmental risk of lead exposure is low during the breeding season. When these migratory eagles reach their winter ranges, however, they likely ingest lead during and soon after the hunting season.

Our objective was to test whether BLL in golden eagles depended on age, sex, year of sampling, or migratory behavior in the Bitterroot Valley, western Montana. We hypothesized that adult and nonmigratory golden eagles would have the highest BLL because they likely had more opportunities to ingest lead from offal piles and carcasses than younger and migratory birds. Additionally, we expected the prevalence of lead-contaminated tissue to be most common during and directly following the general rifle season. Consequently, we predicted golden eagles would tend to have higher BLL after the hunting season.

STUDY AREA

The Bitterroot Valley in the northern Rocky Mountains of western Montana spans roughly 7,500 km^2, with elevations ranging from about 1,000 m to 3,000 m. Lower elevations of the valley are composed mostly of privately owned grasslands and agricultural fields. The uplands and mountains are mainly public land and often consist of mixed conifer forest. Most of this terrain falls within the Bitterroot and Lolo national forests and includes 2 wilderness areas (Welcome Creek, Selway-Bitterroot). The Bitterroot Valley experiences 4 distinct seasons, with big game hunting that occurs on private and public lands for deer (*Odocoileus* spp.) and elk (*Cervus canadensis*) in the fall. Hunting is concentrated during a general rifle season that begins in mid to late October and ends in late November. On some private lands, the hunting season extends from mid-August until mid-February. Between 2011 and 2018, hunters annually harvested an average of 756 elk and 2,069 deer with rifles in the Bitterroot Valley (Montana Fish, Wildlife, and Parks, unpublished data). Carrion from harvested deer and elk is often left in the field as offal piles, bones, blood-shot meat, and un-retrieved animals. Cold temperatures toward the end of the season may freeze carrion or cover it with snow, potentially leaving it to be consumed after it thaws or becomes exposed (Neumann 2009).

METHODS

Capture and Transmitters

Between late November and mid-March from 2011 to 2018, we captured golden eagles on private property using carcasses of road-killed ungulates and mini-net launchers (Trapping Innovations, Jackson Hole, WY, USA). We determined the sex of golden eagles based on morphological characteristics (Bortolotti 1984, Edwards and Kochert 1986) and categorized age based on plumage (Bloom and Clark 2001). We considered birds to be juveniles if we captured them during their first winter. Immature golden eagles were between 2 and 4 years old, and adults were ≥5 years old.

We outfitted mostly immature and adult golden eagles with 45-g or 70-g Solar Argos global positioning system (GPS) transmitters (Microwave Telemetry, Columbia, MD and GeoTrak, Apex, NC, USA). We fastened the transmitters to the birds using a cross-chest, break-away harness made of Teflon ribbon. We programmed transmitters to record 10–15 GPS points daily during daylight hours and 1 point at night. We used the GPS data to identify migratory behaviors. We prepared and uploaded data to Movebank (www.movebank.com, accessed 15 Nov 2018). We did not have BLL for 2 of the golden eagles that received GPS transmitters. The capturing and sampling of golden eagles was in compliance with the University of Montana Institutional Animal Care and Use Committee Animal Use Protocol 046-16 and Federal United States Geological Survey Bird Banding Permit 23353.

Blood Sampling and Lead Analysis

We sampled blood (2–4 mL) from the brachial vein using sterile syringes with 25-gauge needles. We stored samples in glass collection tubes (1.5–2 mL) containing the anticoagulant tripotassium ethylenediaminetetraacetic acid (K_3 EDTA). We measured BLL on a subsample of blood on-site using anodic stripping voltammetry (ASV; LeadCare® 2; Magellan Diagnostics, Chelmsford, MA, USA). Voltammetry allows rapid measurements, although the results lack the accuracy of the more robust spectrometry methods we later used (Langner et al. 2015, Herring et al. 2018). We ran 2 calibration standards provided by the manufacturer at least once for every 10 samples measured. These standards were designed to mimic blood and had been spiked with known concentrations of lead. In all cases, the lead concentration for each standard was within the range of the kit's accuracy. We refrigerated (~4°C) the remaining blood samples for further analysis.

The Environmental Biogeochemistry Lab at the University of Montana (Missoula) digested a subset of blood samples and analyzed them for lead using inductively coupled plasma mass spectrometry (ICP-MS; Langner et al. 2012). The detection limit of the ICP-MS was 0.05 μg/dL. The Louisiana Animal Disease Diagnostic Laboratory (Baton Rouge, Louisiana, USA) analyzed the remainder of the blood samples for lead using atomic absorption spectroscopy (AAS). We sent the second set of samples to a different lab because the ICP-MS from the first lab had become temporarily unavailable. Both the ICP-MS and AAS provide accurate and precise measurements (Herring et al. 2018). Samples were diluted with a 0.1% Triton X-100 solution, acidified with 35% HNO_3, digested

at 85°C, and cooled to ambient temperature. Technicians analyzed digested samples by AAS (PerkinElmer AAnalyst 800, Waltham, MA, USA), with a detection limit for lead of 0.075 μg/dL.

Statistical Analysis

We extracted movement data from Movebank and used the move package in Program R (version 3.5.1, www.r-project.org, accessed 2 July 2018) for subsequent analysis (Kranstauber et al. 2018). Many golden eagles that breed south of 55°N do not migrate (Kochert et al. 2002). We used that latitude to designate whether a golden eagle was migratory or nonmigratory based on GPS data. We removed aberrant GPS points that appeared to have been an error with the GPS transmitter. In these cases, a single location was recorded that implied a rate of travel >200 km/hr, often in a random direction. We created and analyzed all graphics and statistics in RStudio (version 1.0.143, www.r-project.org, accessed 7 Sep 2018).

To compare BLL between age, sex, year of sampling, and migratory behavior, we first checked the distributions for normality using a Shapiro test. All data were right-skewed, so we then log-transformed the distributions, and if data were distributed normally, we compared BLL using a 1-way analysis of variance (sex). Otherwise, we used a Kruskal-Wallis (year of sampling) or a Wilcoxon rank sums test (migratory nature). We found no difference in BLL among years, so we pooled the years. Because distributions of BLL were right-skewed, we compared BLL among ages using a gamma regression and a Tukey *post hoc* test. The model included age class and day of the year as predictors. We used the multcomp package in Program R to test for differences among age classes (Hothorn et al. 2008). We also used the model to test whether BLL tended to decrease over the trapping season for golden eagles. The final model included an identity link function without an interaction term between the predictors because this model had produced the lowest Akaike's Information Criterion (AIC = 790.8). Lastly, we used Pearson's correlation to further investigate whether the log-transformed BLL negatively correlated with time during the trapping season for each age class.

Three golden eagles had measurements from ASV, but there was insufficient blood to measure BLL with ICP-MS or AAS. We used a linear model to estimate BLL for these golden eagles (Fig. S1, available online in Supporting Information). We had 78 samples that had values from both ASV and spectroscopy (from both ICP-MS and AAS). Because ASV only reports BLL between 3.3 μg/dL and 65 μg/dL, some samples had BLL out of range.

RESULTS

We outfitted 2 juvenile, 7 immature, and 20 adult golden eagles with GPS transmitters. Of these, 20 were migratory, and 9 were nonmigratory (Fig. 1). Most migratory golden eagles summered in Alaska ($n = 15$), and the rest summered across the Yukon ($n = 3$), British Columbia ($n = 1$), and Northwest Territories, Canada ($n = 1$). The average summer locations for migratory birds were ≥1,500 km from the capture location. In contrast, the average location for 7 of the 9 nonmigratory golden eagles were within 150 km of the capture location for the remainder of the year (Fig. 1).

The majority of the golden eagles captured were adults (49.5%; $n = 45$), followed by juvenile (27.5%; $n = 25$) and immature birds (23.1%; $n = 21$; Table 1). We caught nearly the same number of adult males as females ($n = 22$ and $n = 23$, respectively). Of juvenile and immature birds, males ($n = 17$ and $n = 12$, respectively) outnumbered females ($n = 8$ and $n = 7$, respectively). We could not determine the sex of 2 immature golden eagles.

Of the 91 golden eagles we caught over 8 winters, 94.5% of them had BLL exceeding concentrations considered background (<10 μg/dL; Table 1; Neumann 2009, Bedrosian et al. 2012, Langner et al. 2015). Four of the 5 birds that did not have elevated BLL were juvenile birds. We saw clinical signs of lead exposure (60–100 μg/dL) in 5 (5.5%) of the golden eagles and acute exposure (>100 μg/dL) in 3 (3.3%) of them. When we pooled data for all birds, the median BLL was 28 μg/dL (SD = 30.9 μg/dL; max. = 252.9 μg/dL). Blood lead levels did not strongly differ among years of sampling ($H = 11.79$, $P = 0.108$) or between the sexes ($F = 0.207$, $P = 0.813$). Blood lead levels tended to be lower in juvenile birds compared to adults ($Z = -2.22$, $P = 0.067$; Table 2; Table S1, available online in Supporting Information).

Blood lead levels decreased over the trapping season for golden eagles ($t = -2.92$, $P = 0.004$; Table 2; Fig. 2A). We tested for an interaction between the day of year and age class but found it to be an uninformative parameter in terms of the model's AIC. We observed some evidence, however, that BLL negatively correlated with the day of year for juvenile and immature birds (Figs. 2B,C). That trend was weaker for adults (Fig. 2D). Blood lead levels did not differ between migratory and nonmigratory golden eagles ($W = 103$, $P = 0.556$, $n = 27$). The relationship between lead concentrations measured by ASV and spectrometry (Fig. S1) was comparable to what Herring et al. (2018) reported for bald eagles (*Haliaeetus leucocephalus*) and California condors.

DISCUSSION

Golden eagles are susceptible to ingesting residual lead fragments from hunted animals. If hunting occurs where golden eagles winter, both migratory and nonmigratory golden eagles may be exposed to lead. We captured 91 golden eagles on their winter habitat in western Montana and nearly all of them had elevated BLL (94.6%; Table 1). This indicates the majority of the birds are likely experiencing the sub-lethal effects of lead exposure. We expected nonmigratory golden eagles to have higher BLL than migratory birds because they might more frequently scavenge shot ground squirrels and prairie dogs, but BLL did not differ between nonmigratory and migratory birds ($n = 27$). Overall, our results show that lead exposure is widespread in golden eagles that winter in western Montana.

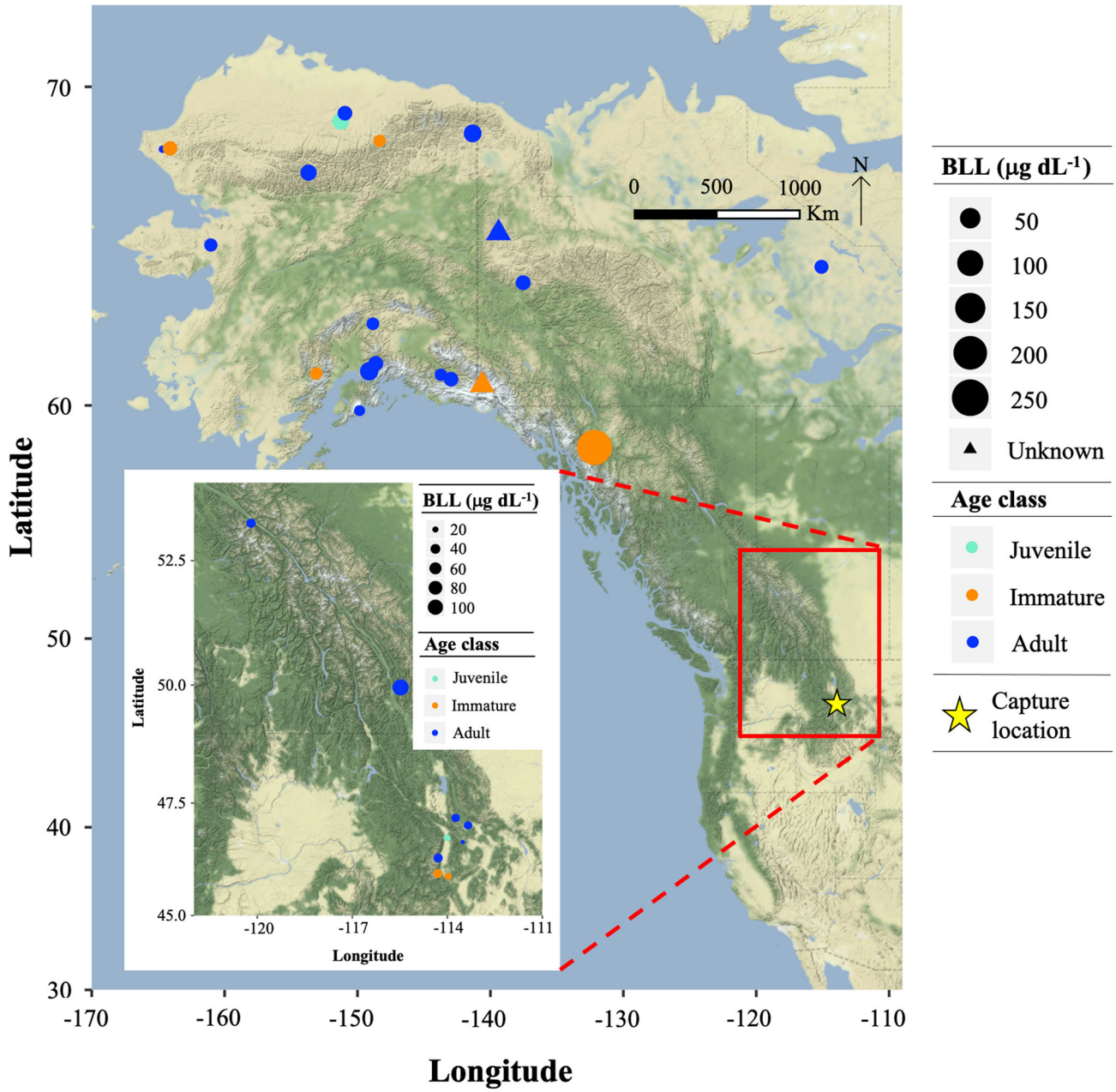

Figure 1. Map showing the average of global positioning system locations where migratory golden eagles spent the summer and nonmigratory golden eagles spent the year (inset) after capture in the Bitterroot Valley, Montana, USA ($n = 29$), 2011–2018. The sizes of the dots are scaled to represent the blood lead level (BLL) of each golden eagle at the time of capture. For the golden eagles represented by triangles, we had insufficient blood to accurately test their BLL. We captured golden eagles from late November to mid-March.

The disparity in BLL between golden eagles in our study and those in past studies may result from various factors, including different seasons of sampling or different big game harvest rates. In southwestern Montana, Harmata and Restani (2013) reported that 45% of golden eagles had BLL >20 µg/dL, whereas 63% of eagles in our study had BLL greater than that threshold. Their study started and ended a month later than ours, potentially limiting the capture of

Table 1. The percentage and number (*n*) of golden eagles for each age class that had blood lead levels in 4 exposure categories. We caught golden eagles between late November to mid-March in the Bitterroot Valley, Montana, USA, 2011–2018. We based categories for blood lead levels on Neumann (2009), Bedrosian et al. (2012), and Langner et al. (2015).

	Background <10 µg/dL	Sub-clinical exposure 10–59 µg/dL	Clinical exposure 60–100 µg/dL	Acute exposure >100 µg/dL
Juvenile	16 (4)	76 (19)	4 (1)	4 (1)
Immature	0	95.2 (20)	4.8 (1)	0
Adult	2.2 (1)	86.7 (39)	6.7 (3)	4.4 (2)

Table 2. Results from a gamma regression that used blood lead levels of golden eagles as a response with day of the year and age class as predictors. We captured golden eagles between late November to mid-March each year in the Bitterroot Valley, Montana, USA, 2011–2018.

	Estimate	SE	*t*	*P*
Intercept	55.326	7.701	7.184	<0.001
Day of the year	−0.272	0.093	−2.921	0.004
Juvenile golden eagle	−13.361	6.013	−2.222	0.029
Immature golden eagle	−8.713	6.800	−1.281	0.204

golden eagles that had recently scavenged carrion that contained lead. Similarly, Langner et al. (2015) reported that 58% of golden eagles migrating along Montana's Rocky Mountain Front in fall had BLL >10 μg/dL (Langner et al. 2015). At that time, the general rifle hunting seasons in Montana were just beginning. In contrast, our study began within 2 weeks of the general hunting season ending, and thousands of animals are killed each year in our study area (Montana Fish, Wildlife, and Parks, unpublished data). This abundance of carrion creates an opportunity for golden eagles to repeatedly consume lead before being captured. In a similar study, but with bald eagles, Bedrosian et al. (2012) observed a higher rate of elevated BLL in Jackson Hole, Wyoming, USA. Bedrosian et al. (2012) reported that during the hunting season, 98% of the bald eagles had elevated BLL, with 25% of those having acute lead exposure (>100 μg/dL). That study, combined with ours, builds evidence that BLL in golden and bald eagles may be highest during and directly following the hunting season.

Golden eagles caught later in the trapping season tended to have lower BLL than those captured earlier (Fig. 2A; Table 2). This negative relationship between BLL and day of year appeared most pronounced for juvenile and immature birds (Figs. 2B,C). As time passed after the end of the general hunting season, we expected a lower availability of carrion that contained lead. But some carcasses could still have been available from supplemental hunting, shot livestock or coyotes (*Canis latrans*; Stauber et al. 2010), or after snow melted off previously covered carcasses (Neumann 2009). Additionally, BLL should have decreased because the half-life of lead is often considered to be 14 days after exposure based on work with California condors (Fry et al. 2009). Even though young birds tend to outcompete adults at carcasses (Gjershaug et al. 2019), BLL remained high across the season for adults (Fig. 2D). Adults tend to have more lead in their bones than younger birds (Slabe 2019), and that lead can reenter blood over time (Rabinowitz et al. 1976), potentially preventing BLL from decreasing to pre-exposure levels. Lastly, adults may find carrion more easily than younger birds that lack similar experience.

Our capture of free-flying golden eagles likely produced a more representative sample of BLL than studies reliant on golden eagles admitted to veterinarians or rehabilitation centers (Wayland and Bollinger 1999, Stauber et al. 2010). Rehabilitation centers often receive injured or emaciated eagles, representing a biased sample of the general population. But we cannot rule out bias in our study. Langner et al. (2015) reported that golden eagles captured on carrion in the fall had higher BLL than golden eagles caught on live bait. We may have avoided some of this potential bias by capturing eagles in winter. In winter, golden eagles may feed more heavily on carrion when live prey, such as ground

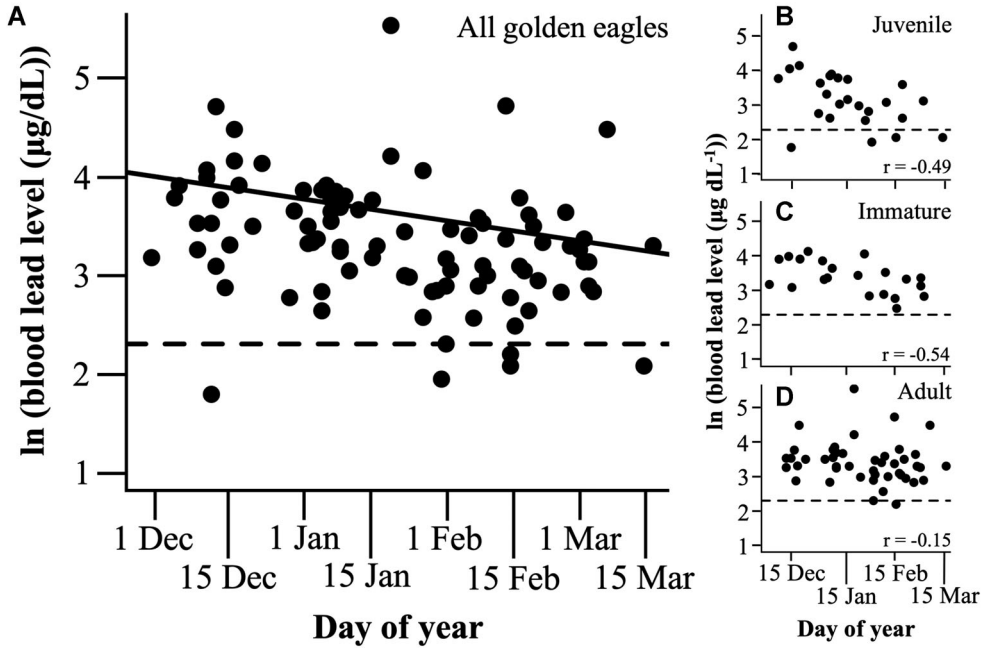

Figure 2. Blood lead levels of all golden eagles (A) and by age class (B–D). The dashed line represents the threshold for elevated blood lead levels (10 μg/dL). The r-values on B–D resulted from a Pearson's correlation. We captured golden eagles from late November to mid-March in the Bitterroot Valley, Montana, USA, 2011–2018.

squirrels and marmots (*Marmota* spp.), are less common (Kochert et al. 2002).

Our results indicate that nonmigratory and migratory golden eagles are being exposed to lead (Fig. 1). Although mortalities from lead poisoning are often reported at veterinary clinics and rehabilitation centers (Wayland and Bollinger 1999, Stauber et al. 2010, Cruz-Martinez et al. 2012), lead poisoning has not been linked to population-level declines of golden eagles. The trajectories of some populations of golden eagles appear stable, whereas others show potential declines (Millsap et al. 2013, 2016; Sherrington, Rocky Mountain Eagle Research Foundation, unpublished reports). Various anthropogenic threats besides lead poisoning may contribute to these declines, such as habitat loss, reductions in prey base, vehicle collisions, electrocutions, and industrial wind energy. Lead exposure at sublethal levels may impair golden eagles enough to make them more susceptible to these types of fatality (Haig et al. 2014).

MANAGEMENT IMPLICATIONS

Lead exposure affects most golden eagles that winter and breed in western Montana, regardless of migratory behavior. Unlike other threats to golden eagles, like habitat loss and energy development, that require the cooperation of many stakeholders to find a solution, the solution to lead exposure is simple: hunters can use nonlead ammunition. To accelerate a transition to nonlead, state agencies, sportsman groups, and wildlife organizations should run outreach programs, such as hunter education classes, that encourage hunters to voluntarily switch. These efforts will be most effective in areas that have intense hunting and high densities of golden eagles.

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