Feather Lead Concentrations and $^{207}Pb/^{206}Pb$ Ratios Reveal Lead Exposure History of California Condors (*Gymnogyps californianus*)

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Lead poisoning is a primary factor impeding the survival and recovery of the critically endangered California Condor (*Gymnogyps californianus*). However, the frequency and magnitude of lead exposure in condors is not well-known in part because most blood lead monitoring occurs biannually, and biannual blood samples capture only ~10% of a bird's annual exposure history. We investigated the use of growing feathers from free-flying condors in California to establish a bird's lead exposure history. We show that lead concentration and stable lead isotopic composition analyses of sequential feather sections and concurrently collected blood samples provided a comprehensive history of lead exposure over the 2−4 month period of feather growth. Feather analyses identified exposure events not evident from blood monitoring efforts, and by fitting an empirically derived timeline to actively growing feathers, we were able to estimate the time frame for specific lead exposure events. Our results demonstrate the utility of using sequentially sampled feathers to reconstruct lead exposure history. Since exposure risk in individuals is one determinant of population health, our findings should increase the understanding of population-level effects from lead poisoning in condors; this information may also be helpful for other avian species potentially impacted by lead poisoning.

Introduction

California Condors, currently listed as endangered by the United States Federal Government (*1*) and critically endangered by the International Union for Conservation of Nature (IUCN) (*2*), are obligate scavengers. Studies have indicated that lead poisoning from ingestion of spent ammunition fragments embedded within animal carcasses shot with lead ammunition remains one of the primary factors limiting the California Condor's survival and recovery (*3–5*). Recent monitoring of free-flying condors in California (~80 individuals in 2008) found that 38% of 238 collected blood samples exceeded a proposed lead-exposure threshold of 10 μg/dL (*5*), while 12% of samples were above 30 μg/dL (*32*). For reference, the Center for Disease Control's blood lead action level for lead exposure in children is 10 μg/dL (*6*); there is no accepted blood lead action level for lead exposure in wildlife. When necessary, lead-poisoned condors are treated with chelation therapy to reduce the risk of morbidity or mortality; typically, chelation treatment would be considered in condors with blood lead levels ≥30 μg/dL (*33*).

In wildlife, as with humans, morbidity and mortality from lead poisoning is associated with an individual's lead exposure history. Thus, condors with a higher frequency of elevated exposures are at higher risk for morbidity compared to birds with lower exposures. In order to link lead exposure to annual survivorship rates and ultimately to population health, estimating an individual's lead exposure risk on an annual basis is important (*3, 7*). Blood lead levels, while indicative of recent exposure, may be of limited use to assess annual exposure risk in California Condors since lead is relatively rapidly cleared from blood following an exposure event (estimated elimination half-life ~ 13 days (*8*)). Annual or biannual blood lead monitoring will capture only about 5−10% of a bird's annual exposure history. Consequently, establishing comprehensive lead exposure biomarkers for condors as well as other avian species (e.g., eagles (*9*)) considered at high risk for lead exposure is needed.

Feather samples can be collected from live birds, and sampling does not require the collection of the entire feather. Furthermore, feathers have already shown promise as a biomarker for lead exposure in avian species (*10–13*), though those studies did not explore whether subsections within a feather may capture a history of lead exposure over the period of feather growth. In a previous study (*4*) we reported lead concentrations and stable isotopic compositions in sequential subsections of a feather collected postmortem from a condor that died from lead poisoning. Analysis of feather subsections illustrated the acute lead exposure event that led to the bird's death and suggested that feathers may capture a bird's lead exposure history over the prolonged period of feather growth (*4*).

Here, we move beyond our preliminary investigations to demonstrate the utility of sequential sampling of a feather vane along the length axis of growing feathers as a biomarker of lead exposure history in free-flying California Condors. We analyzed condor feather and blood samples for lead concentrations and stable lead isotopic compositions to investigate the frequency, magnitude, and source of lead exposure over the preceding months of feather growth. Since lead exposure risk in individuals is one determinant of

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population health (*3, 7*), our findings should increase the understanding of population-level effects from lead poisoning in condors, as well as other avian species such as bald eagles, golden eagles, and the globally threatened Spanish imperial eagle (*9, 13*).

Materials and Methods

Study Subjects. We measured lead concentrations and stable isotopic compositions in tissue samples from six free-flying condors in California that represent seven case studies as detailed below and grouped based on (1) a well-documented lead exposure event, (2) mortalities associated with lead poisoning, and (3) routine lead exposure monitoring.

(1) Documented Lead Exposure Event Involving Condors 306 and 318. On the 24/25th of June, 2007, California Condors 306 and 318 were observed feeding on (318) or in the near vicinity of (306, based on GPS satellite tracking) the carcass of a pig shot several miles east of Pinnacles National Monument (PNM). The pig carcass was recovered by PNM biologists and X-rayed, which identified a classic 'snowstorm' of radio-opaque fragments within the cranium, along with two intact bullets in the chest and gut region; the intact bullets were subsequently recovered for analyses. Because of concern over possible lead exposure, several condors that were in the vicinity of, or observed feeding on, the pig carcass (including 306 and 318) were trapped on the 2nd of July, 2007, for exposure assessment. The field-based blood results (Lead-Care, Long Island City, NY) for condors 306 and 318 indicated lead poisoning, with blood lead levels >50 μg/dL. A whole blood sample was collected for lead concentration and isotopic composition analyses, and condors 306 and 318 were transported to the veterinary facility at the Los Angeles (LA) Zoo for clinical management and chelation therapy (treatment administered over the 3rd to 16th of July). A second blood sample was collected on the 29th July, 2007, after the birds had been returned to PNM and prior to release back into the wild; at the same time, 18 feather vane samples were collected from the growing left fifth primary flight feather of condor 306 (total length of feather vane 42.5 cm), and 16 feather vane samples were collected from a primary flight feather (exact feather number not recorded) of condor 318 (total length of feather vane 37.5 cm) for lead concentration and isotopic composition analyses.

(2) Lead Poisoning and Mortality of Condors 336 and 238. In early September, 2008, condor 336 was observed perched in a tree in Big Sur, CA, severely moribund. On the 5th of September, 2008, the bird fell out of the tree and was immediately recovered by PNM and Ventana Wildlife Society (VWS) condor biologists. A collected whole blood sample indicated lead poisoning (>50 μg/dL), based on field-based measurements; subsequent laboratory-based measurements determined the blood lead level was ~150 μg/dL (Louisiana Animal Disease Diagnostic Laboratory). The bird was transported to the LA Zoo where she died two days later (7th of September, 2008). The carcass was shipped to the Zoological Society of San Diego where postmortem liver, kidney, and a section of tibiotarsus were collected for lead concentration and isotopic composition analyses. The entire growing right primary feather no. 9 (37.5 cm total length) and the right retrix feather no. 3 (24.4 cm total length) were also collected.

On the 4th of May, 2008, condor 238 was trapped by US Fish and Wildlife Service (USFWS) condor biologists at the Bitter Creek National Wildlife Refuge (California) for routine monitoring purposes. A whole blood sample collected on the 6th of May, 2008, indicated lead poisoning, with an elevated field-based value and a subsequently determined laboratory-based value of 74 μg/dL (California Animal Health and Food Safety). The bird was immediately transported to the LA Zoo where he died on the 10th of May, 2008. The carcass was shipped to the Zoological Society of San Diego where postmortem liver, kidney, and a section of tibiotarsus were collected for lead concentration and isotopic composition analyses. An entire growing primary feather (36.2 cm total length, exact primary feather not recorded) was also collected.

(3) Routine Lead Monitoring of Condors 307, 336, and 351. Whole blood and growing primary feather vane samples were collected from condors 307, 336 (collected December, 2006; note this bird later died from lead poisoning in September 2008; see above), and 351 during routine lead exposure monitoring efforts. Blood was collected from condor 307 on the 3rd of July, 2006, and a field-based blood test indicated lead poisoning with a blood lead level of ~47 μg/dL. This bird was then transported to the veterinary facility at the LA Zoo for clinical management and chelation therapy and returned to PNM. Prior to rerelease, 13 feather vane samples were collected from the growing left sixth primary flight feather (length of feather vane 27 cm) by PNM biologists on the 20th of July, 2006. For condor 336, blood and 13 feather vane samples were collected from the growing left 10th primary feather (length of feather vane 26 cm) by PNM biologists on the 14th of December, 2006. Blood and 16 feather vane samples were collected from the growing right seventh primary feather of condor 351 (length of feather vane 32 cm) by VWS biologists on the 2nd of November, 2007.

Feather Growth Rate Assessment. Since the ability to reconstruct condor lead exposure history through the analyses of growing feather vane sections depends in part on an accurate estimate of feather growth rates, we determined growth rates for primary, secondary, and tertiary condor flight feathers using feather growth bars as a marker of daily feather growth. The use of feather growth bars to determine rates of feather generation is well-established in other avian species, as these growth bars are visible with the naked eye and are measurable indices of daily feather production (*14–17*).

We measured feather growth bars using digital calipers from 20 feathers ($n = 12$ primary, 2 secondary, and 6 tertiary) collected opportunistically by USFWS, PNM, and VWS biologists after feathers were molted from free-flying condors. We determined an average growth rate of primary flight feathers of 4.4 ± 0.28 mm/day (total sample pooled variance of both within and between feather measurements; $n = 12$, range = 4.1−4.8 mm/day). Overall, the average growth rate across the three feather types we evaluated (primary, secondary, and tertiary flight feathers) was 4.4 ± 0.39 mm/day (total sample variance; $n = 20$, range = 3.5−5.3 mm/day). These average feather growth rate values agree well with California Condor primary feather growth rates (4.3−6.0 mm/day) estimated by monitoring feather molt patterns in free-flying condors (*18*). Although we are confident of the feather growth rate derived from measuring growth bars in molted condor feathers, we recognize that further study is needed to evaluate whether feather growth rates are significantly different under conditions of prolonged environmental and physiological stress. It is noteworthy that the growth bar measurements for condor 336, who died following a period of prolonged lead poisoning, indicated no change in feather growth rate over this lead exposure period.

Sample Collection. Whole blood samples (1−3 mL) were collected from the tibial artery directly into low-lead Vacutainers (Fisher Scientific, Pittsburgh, PA) using a 19- or 21-gauge catheter, as previously described (*4*). Liver, kidney, and tibiotarsus were collected postmortem from condors 238 and 336 by veterinary pathology staff at the Zoological Society of San Diego and stored in plastic cryovials at −20 °C until processing for analyses.

Feather vane samples from growing primary flight feathers were collected from condors 306, 307, 318, 336 (14th of December, 2006, collection), and 351 while birds were

restrained for blood collection. Growing primary flight feathers were identified by condor biologists; sections of feather vane measuring ~2 × 2 cm were trimmed sequentially along the trailing edge of the feather (Supporting Information (SI) Figure S1), placed in individual ziplock plastic bags, and stored at room temperature until processed for analyses.

Intact whole growing feathers were collected postmortem from condors 336 and 238 by pulling the feather out of the feather follicle; intact feathers were stored in polyethylene bags at room temperature until processed for analyses. Within the trace metal clean laboratory, the calamus sheath was removed to expose newly grown feather vane, and sequential ~2 cm vane sections were collected by cutting along the vane−rachis junction using stainless steel scissors; the calamus section, void of feather vane (SI Figure S1), was also collected in 2 cm sections for analyses as our previous studies showed lead concentration and isotopic agreement between condor feather vane and associated rachis tissue (*4*).

Sample Processing. All biological samples (feather, blood, soft tissue, bone) were processed and analyzed using established trace metal clean techniques under HEPA filtered air laboratory conditions, following procedures previously described (*19–21*). Individual sections of feather vane (or rachis) were treated as separate samples. Each feather section was weighed, rinsed sequentially with HPLC grade methanol, ultrapure water, 1% HNO_3, and rinsed a final time with ultrapure water. Whole blood, liver, kidney, and bone samples were processed and analyzed as described previously (*4, 20, 22*). Spent ammunition bullets recovered from the pig carcass ($n = 2$) were cleaned by rinsing sequentially with HPLC grade methanol, 1% HNO_3, and ultrapure water. The individual bullets were then leached in 2 mL of 1% HNO_3 for 30 s.

Sample Analysis. Lead concentrations and isotopic ratios were determined using a Finnigan MAT Element magnetic sector−inductively coupled plasma mass spectrometer (ICP-MS), measuring masses of ^{206}Pb, ^{207}Pb, ^{209}Bi, and/or ^{205}Tl (the latter as internal standards), as previously described (*22, 23*) (see also SI 1). The precision (2× relative standard deviation, 2 RSD) of lead isotopic measurements was 0.27% or better for $^{207}Pb/^{206}Pb$ ratios, based on repeated measurements (≥3) of condor blood, kidney, bone, and feather samples. Long-term precision for a previously digested blood sample measured over the course of the sample analysis (31st of January, 2007−19th of December, 2008) was 0.20% (2 RSD) for $^{207}Pb/^{206}Pb$ ratios. The precision of lead concentration measurements was better than 1.5% (2 RSD) (See SI 1 for more detail on intercalibration and precision measurements.)

GPS Satellite Telemetry Data for Condor 238. Condor 238 was equipped with a solar-powered GPS satellite transmitter (Microwave Telemetry, Inc., Columbia, MD) attached to a patagial tag, from the 21st of September, 2007, until he was trapped on the 4th of May, 2008. The transmitter was programmed to transmit GPS location data to satellites hourly between 05:00−20:00 (PST); GPS data were calculated and distributed by CLS America, Inc. (Largo, MD). GPS satellite transmitter data were plotted and quantified using ArcMAP GIS software (ESRI, Redlands, CA) and were used to evaluate space use and movement patterns for condor 238 during the estimated timeline of exposure derived via sequential feather sampling as well as the two weeks prior to capture (4th of May, 2008).

Results and Discussion

(1) Documented Lead Exposure Event Involving Condors 306 and 318. Blood and feather $^{207}Pb/^{206}Pb$ ratios and lead concentrations from condors 306 and 318 clearly illustrate acute lead exposure to spent ammunition from the recovered pig carcass. To our knowledge, this is the first documented link between ammunition in a hunter-killed carcass in California and elevated lead levels in condor blood and feathers. Lead levels in blood collected ~1 week after feeding on the pig carcass were 146 and 80 μg/dL for condors 306 and 318, respectively, with $^{207}Pb/^{206}Pb$ ratios that matched the $^{207}Pb/^{206}Pb$ ratios of the bullets recovered from the pig (Figure 1, SI Table S1). Notably, the $^{207}Pb/^{206}Pb$ ratio of the recovered lead bullets (0.8170) falls within the upper range of $^{207}Pb/^{206}Pb$ ratios in ammunition measured by Church et al. (*4*) (i.e., ~0.805−0.818). Approximately 1 month later, following chelation treatment at the Los Angeles Zoo, blood lead levels had declined to ~15 μg/dL in both birds, with $^{207}Pb/^{206}Pb$ ratios that were slightly higher than the values in the prechelation blood samples (Figure 1A). As expected, these blood data fit a two end-member mixing model, with the equation $Y = (3.705 \cdot 0.8418 + (X - 3.705)0.8170)/X$, where the "background" lead end-member is represented by the average blood lead level (3.705 μg/dL) and $^{207}Pb/^{206}Pb$ ratio (0.8418) in condors ($n = 9$) at PNM prior to being released into the wild for the first time, and the exposure source end-member is represented by the $^{207}Pb/^{206}Pb$ ratio of the spent lead ammunition recovered from the pig carcass (0.8170). The pre- and postchelation blood $^{207}Pb/^{206}Pb$ ratios of condors 306 and 318 fall on or within the isotope ratio measurement error of the mixing line (Figure 1A).

Sequential feather vane samples collected over the length of feather growth illustrate the lead exposure history for condors 306 and 318 (Figure 1B and C; SI 2). The lead concentrations and $^{207}Pb/^{206}Pb$ ratios in feather vane segments from condor 306 (Figure 1B) show that, prior to exposure, feather lead levels were low (<0.3 μg/g) with an isotopic signature reflective of prerelease (i.e., nonlead-exposed) condors at PNM ($^{207}Pb/^{206}Pb = 0.836$ to 0.848, $n = 9$). Immediately following exposure on the 25/26th of June, 2007, feather lead levels sharply increase while feather $^{207}Pb/^{206}Pb$ ratios decrease to match the isotopic signature of the recovered lead ammunition from the pig carcass and the bird's blood collected soon after exposure (Figure 1B).

Data for condor 318 (Figure 1C) show a similar though less dramatic pattern, with feather lead levels sharply increasing while the $^{207}Pb/^{206}Pb$ ratios change to match the isotopic signature of the recovered lead ammunition from the pig carcass as well as the bird's blood collected soon after exposure. Data from this feather also provide evidence for a lead exposure event prior to initiation of feather growth (i.e., >80 days before feather sample collection). The source of this prior exposure is not known, though the $^{207}Pb/^{206}Pb$ signature appears consistent with the $^{207}Pb/^{206}Pb$ ratios of ammunition reported by Church et al. (*4*). As predicted, lead concentrations in the most recently grown feather vane segments from condors 306 and 318 demonstrate the beginning of a sharp decline, consistent with a rapid reduction in blood lead levels following chelation treatment.

(2) Lead Poisoning and Mortality of Condors 336 and 238. Feathers combined with tissue samples (bone, liver, kidney, and blood) present a detailed exposure history for condors 336 and 238 (Figure 2, SI Table S2), who died from lead poisoning (336) or from undetermined causes following treatment for lead poisoning (238). First, whole blood collected from condor 336 shortly before death had a lead concentration of ~94 μg/dL with an unusual $^{207}Pb/^{206}Pb$ ratio of 0.9164, while liver and kidney tissue collected postmortem had very elevated lead levels of ~48 and 130 μg/g (dry wt) and similarly unusual $^{207}Pb/^{206}Pb$ ratios of 0.9179 and 0.9174, respectively (SI Table S2). Tibiotarsus bone was also elevated in lead (~15 μg/g dry wt) with a $^{207}Pb/^{206}Pb$ signature of 0.8649, somewhat intermediate between the blood and soft tissues and the background $^{207}Pb/^{206}Pb$ signature in prerelease condors from PNM (~0.842); this likely reflects the relatively slower uptake of lead into bone compared to well-perfused tissues like liver and kidney following an exposure event (*24*).

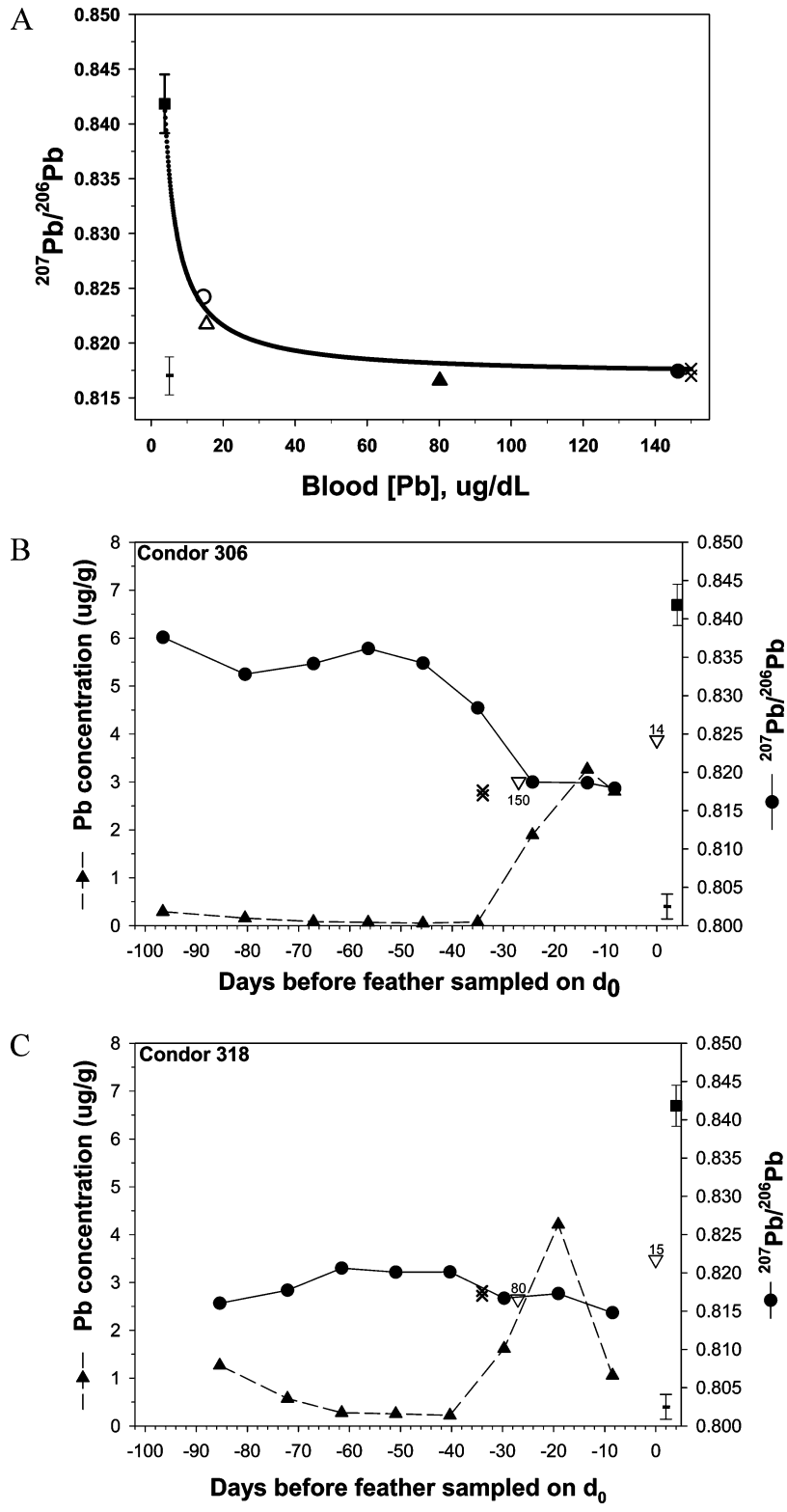

FIGURE 1. (A) Lead concentrations versus $^{207}Pb/^{206}Pb$ ratios in blood collected on the 2nd of July, 2007, from condors 306 (●) and 318 (▲) several days following documented lead exposure and again on the 29the of July, 2007 (condors 306 (○) and 318 (△)) after chelation treatment. Also shown is the average blood lead concentration and $^{207}Pb/^{206}Pb$ ratio in prerelease condors at PNM (■ ± 2 SE, $n = 9$; blood collected April 2007 and September 2009). The mixing line, derived using the equation $Y = (3.705 \cdot 0.8418 + (X - 3.705)0.8170)/X$, shows the calculated $^{207}Pb/^{206}Pb$ ratio of condor blood containing the $^{207}Pb/^{206}Pb$ signature from prerelease condors (i.e., background lead) mixed with increasing amounts of contaminant lead from the spent ammunition recovered from the pig carcass (×). The ICP-MS measurement error is shown in the lower left (—) ± 2 SD. (B and C) Feather $^{207}Pb/^{206}Pb$ ratios (●) and lead concentrations (▲) versus days before d_0, the date the feather was sampled (29th of July, 2007) for condors 306 and 318 (see SI 2 for an explanation of timeline estimation): (×) $^{207}Pb/^{206}Pb$ ratios of the spent ammunition recovered from the pig carcass; (▽) $^{207}Pb/^{206}Pb$ ratios for blood samples collected prechelation (2nd of July, 2007, d_{-27}) and postchelation treatment (29th of July, 2007, d_0); numbers adjacent to each triangle indicate the blood lead concentration (μg/dL). Also shown (along the right y-axis) is the average blood lead $^{207}Pb/^{206}Pb$ ratio in prerelease condors at PNM (■ ± 2SE, $n = 9$). The ICP-MS measurement error is shown in the lower right (—) ± 2 SD.

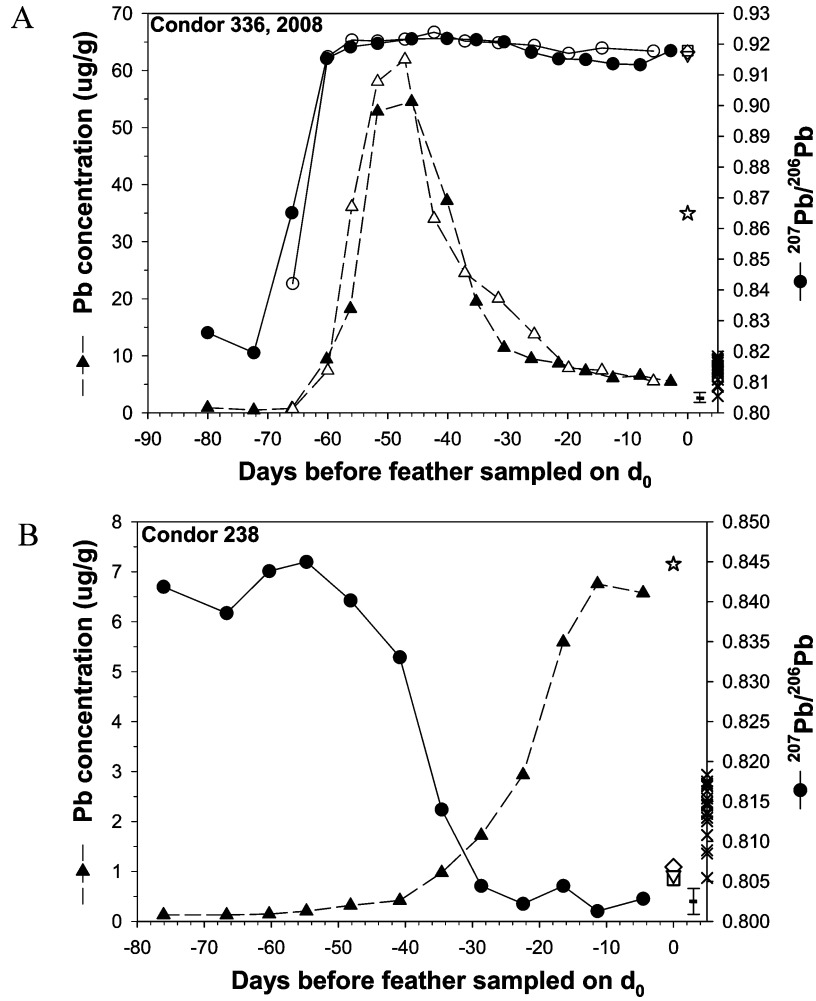

FIGURE 2. (A) Segments from condor 336 primary feather ^{207}Pb/^{206}Pb ratios (●) and lead concentrations (▲) and retrix feather ^{207}Pb/^{206}Pb ratios (○) and lead concentrations (△) versus days before d_0, the date the feathers were sampled (immediately following death). Lead ammunition from California reported by Church et al. (*4*) is represented by × (along the right *y*-axis). ^{207}Pb/^{206}Pb ratios in blood (▽), kidney (◇), and liver (□) collected pre- and postmortem (plotting on top of one another in the upper right) are identical and match the ^{207}Pb/^{206}Pb ratios in feather vane grown after exposure (~d_{-70}). The ^{207}Pb/^{206}Pb ratios in bone (☆) were intermediate between the ^{207}Pb/^{206}Pb ratios of the blood and soft tissues and most distal (pre-exposure, oldest) feather segment. The ICP-MS measurement error is shown in the lower right (—) ± 2 SD. (B) Condor 238 feather ^{207}Pb/^{206}Pb ratios (●) and lead concentrations (▲) in primary feather segments versus days before d_0, the date the feather was sampled (immediately following death). The ^{207}Pb/^{206}Pb signature falls within the range of lead ammunition from California (×, along the right *y*-axis) reported by Church et al. (*4*). ^{207}Pb/^{206}Pb ratios in blood (▽), kidney (◇), and liver (□) collected pre- and postmortem (plotting on top of one another in lower right) are identical and match the ^{207}Pb/^{206}Pb ratios in the feather vane grown after exposure (~d_{-50}), while the bone ^{207}Pb/^{206}Pb ratio (☆) is consistent with values observed in the pre-exposure (oldest) feather segments. The ICP-MS measurement error is shown in the lower right (—) ± 2 SD.

The lead concentrations and ^{207}Pb/^{206}Pb ratios of sequential feather segments from both a primary flight feather and a rectrix (i.e., tail) feather show remarkable agreement and reveal that condor 336 was acutely lead exposed >2 months before the bird died of lead poisoning on the 7th of September, 2008 (Figure 2A). Using a primary feather growth rate of 4.41 mm/day, we estimate that lead exposure occurred between the 21st of June and 1st of July, 2008 (see Materials and Methods; note that for the rectrix feather a growth rate of 3.6 mm/day was used, determined by calibrating to the primary feather). Our estimated timeline of exposure underscores the added value of feather analyses to reconstruct lead exposure histories in condors. The elevated blood lead level (94 μg/dL) of condor 336 determined shortly before death on the 7th of September, 2008, might typically be interpreted to suggest that exposure occurred over the preceding week or two, i.e., mid-August to early September (assuming a blood lead elimination half-life of 13 days in condors (*8*)), which would have been after implementation of the partial ban on lead ammunition in California on the 1st of July, 2008 (*25*). Instead, our data suggest that exposure occurred immediately prior to implementation of the partial lead ammunition ban.

The feather segments indicate that condor 336 was exposed to a lead source with an unusual ^{207}Pb/^{206}Pb signature (~0.92) compared to ^{207}Pb/^{206}Pb ratios in either prerelease birds from PNM (~0.842) or lead ammunition (~0.805 to 0.818) measured by Church et al. (*4*). Further, the feather segment lead concentrations demonstrate that condor 336 suffered far more extreme lead poisoning than would otherwise be concluded from the blood lead level at the time of death (94 μg/dL). Over a period of ~20 days of feather growth, feather lead concentrations sharply increased to ~55 μg/g with exposure, then declined to ~5 μg/g by the time the bird presented with overt morbidity. Notably, these peak feather lead concentrations were more than an order of magnitude higher than those measured in the documented

lead poisoning cases involving condors 306 and 318 (i.e., ~55 versus ~4 μg/g), discussed above.

We estimated that condor 336 had a blood lead concentration at the peak of exposure of ~1100 μg/dL, based on the measured peak feather lead levels of ~58 μg/g and an estimated blood:feather lead concentration ratio of 19 (SI 3), which is consistent with the observed gross morbidity and eventual mortality; it also helps explain the very elevated kidney and liver lead concentrations measured postmortem (SI Table S2). This extraordinarily high estimated blood lead level is similar to blood lead levels of 900−1300 μg/dL exhibited by Andean condors two weeks after being fed 2−6 lead shot pellets (*26*), supporting our contention that incidental exposure to a concentrated lead source such as spent lead ammunition is a plausible cause of the lead poisoning mortality of condor 336.

The case of condor 238, which resided in the southern California region, is somewhat similar to that described above for condor 336. The lead concentrations and $^{207}Pb/^{206}Pb$ ratios of condor 238's feather segments show a significant lead exposure event that we estimate started between the 12th and 29th of March, 2008, ~50 days before death to a lead source with a $^{207}Pb/^{206}Pb$ signature that falls at the lower range of lead ammunition measured by Church et al. (*4*) (Figure 2B). As with condor 336 above, this estimated timeline of exposure is calculated from our measured average primary feather growth rate of 4.41 mm/day. Using the peak feather lead concentration of 6.8 μg/g, we can estimate a peak blood lead level following exposure of ~130 μg/dL, using the blood: feather lead concentration ratio of 19 noted above (see also SI 3). Interestingly, the rate of onset of lead poisoning in condor 238 appeared more gradual than in condor 336, based on the slower rate of increase in lead concentrations of the feather segments following the first evidence of exposure (i.e., changing feather $^{207}Pb/^{206}Pb$ ratios). For condor 238, peak lead levels in feathers were not reached until ~40 days after exposure (~10 days before death), whereas in condor 336 the estimated interval between exposure and the very elevated peak feather lead levels was only ~20 days, consistent with her extraordinarily acute exposure (Figure 2A and B).

The blood sample collected from condor 238 shortly before death had a lead concentration of 65 μg/dL and a $^{207}Pb/^{206}Pb$ ratio of 0.8058; this $^{207}Pb/^{206}Pb$ ratio matched the more recently grown (proximal) feather segments with the highest lead levels and was consistent with exposure to lead ammunition (*4*). Liver, kidney, and tibiotarsus bone samples were only moderately elevated at ~2−4 μg/g (dry wt), and liver and kidney possessed $^{207}Pb/^{206}Pb$ ratios comparable to blood (i.e., 0.8053 and 0.8068, respectively), while bone was more similar isotopically to background lead in prerelease condors from PNM (~0.842, this study) and Hopper Mountain National Wildlife Refuge (~0.832 (*4*)). Additionally, condor 238's bone lead isotopic composition matched the most distal (oldest) feather segments exhibiting low, pre-exposure lead levels (Figure 2B).

Given that condor 238 was fitted with a GPS satellite transmitter, we explored the potential of using GPS-based spatial location data in combination with lead exposure history information derived from the bird's sequentially sampled feather to constrain the geographic region of lead exposure. Two hundred-forty locations for condor 238 were transmitted to satellites during the estimated time frame of exposure based on the feather data (i.e., 12−29 March, 2008); most GPS locations were on, near, or between the Bitter Creek or Hopper Mountain National Wildlife Refuges (SI Figure S2), where supplemental feeding occurs and condors regularly congregate to forage and roost (*34*). In addition to the use of the National Wildlife Refuges, GPS data indicated that 238 used habitat within San Luis Obispo, Santa Barbara, Kern, and Los Angeles Counties during this period, though with less frequency (SI Figure S2). Comparison of these GPS location data with similar data ($n = 199$ transmitted locations) collected over the 2 weeks prior to capture (i.e., 20th of April−3rd of May, 2008) shows gross differences in location between these two timeframes (SI Figure S2). However, pinpointing the location of lead exposure for condor 238 is not possible due to the limited richness of data currently available via GPS satellite tracking (e.g., location data is only transmitted hourly, and condors are known to arrive at, forage, and depart a carcass within an hour (*35*)), as well as a lack of field-based forensic evidence to verify possible exposure hazards (e.g., carcasses).

(3) Routine Lead Exposure Monitoring of Condors 307, 336, and 351. Feather vane samples collected from condors during routine lead exposure monitoring efforts complement and substantially extend exposure assessments based on a concurrently collected blood sample. Feather lead levels from condor 307 reveal a lead exposure event ~60 days prior to feather sample collection, with an apparent second exposure episode occurring several weeks later (~38 days before feather collection) (Figure 3A, SI Table S3, SI 4). In both instances, the feather $^{207}Pb/^{206}Pb$ ratios suggest that the exposure source(s) had a lead isotopic signature within the range of lead ammunition reported by Church et al. (*4*). The blood sample, which was collected on the 3rd of July, 2006 (i.e., 17 days prior to feather sample collection on the 20th of July, 2006), was moderately high in lead concentration (53 μg/dL) and possessed a $^{207}Pb/^{206}Pb$ ratio that was similar to, but slightly lower than, the most recently grown feather vane sample and, again, within the midisotopic range of lead ammunition (*4*). Noteworthy is that condor 307 was treated for another lead poisoning event in December 2006 and found dead in Big Sur from a rattlesnake bite in May 2007 (*36*).

Feather vane lead concentrations and $^{207}Pb/^{206}Pb$ ratios from condor 336 (collected the 14th of December, 2006; note that this condor died from a subsequent lead exposure event; see above) provide clear evidence of a significant lead exposure event that was not reflected in the concurrently collected blood sample. The blood lead concentration at the time of feather vane collection was 10 μg/dL, with a $^{207}Pb/^{206}Pb$ ratio at the upper range of lead ammunition (*4*). However, the feather vane samples show that this bird was exposed to a very elevated lead source sometime before the onset of feather growth (~70 days before feather collection) (Figure 3B, SI Table S3, SI 4); the most distal (oldest) vane segment exhibited a lead concentration of 7.3 μg/g with a $^{207}Pb/^{206}Pb$ signature within the midrange of lead ammunition (Figure 3B). Using the blood:feather lead concentration ratio of 19 (SI 3), we can estimate that condor 336 had a blood lead value of ~140 μg/dL, which is considered lead poisoned and would have warranted chelation treatment had the exposure been detected.

The blood sample from condor 351 was low in lead concentration (4 μg/dL) and possessed a $^{207}Pb/^{206}Pb$ ratio (0.8291) intermediate between background lead and lead ammunition (*4*). Lead concentrations in the newly grown (proximal) feather vane segments were also very low, consistent with the low blood lead level, but the oldest (distal) vane segments are suggestive of a lead exposure event prior to the onset of feather growth (>90 days before sample collection) (Figure 3C, SI Table S3, SI 4). Interestingly, the $^{207}Pb/^{206}Pb$ ratios in the five newest (proximal) feather vane segments, which we estimate to have grown ~20−40 days prior to collection of the blood sample, isotopically match the blood sample collected on the 2nd of November, 2007. The more distal (older) vane segments evidence a cyclical variation in $^{207}Pb/^{206}Pb$ ratios between ~0.829 (the blood $^{207}Pb/^{206}Pb$) and ~0.839 (i.e., background lead). This isotopic variation is not paralleled by changes in feather vane lead concentrations and cannot be explained by $^{207}Pb/^{206}Pb$

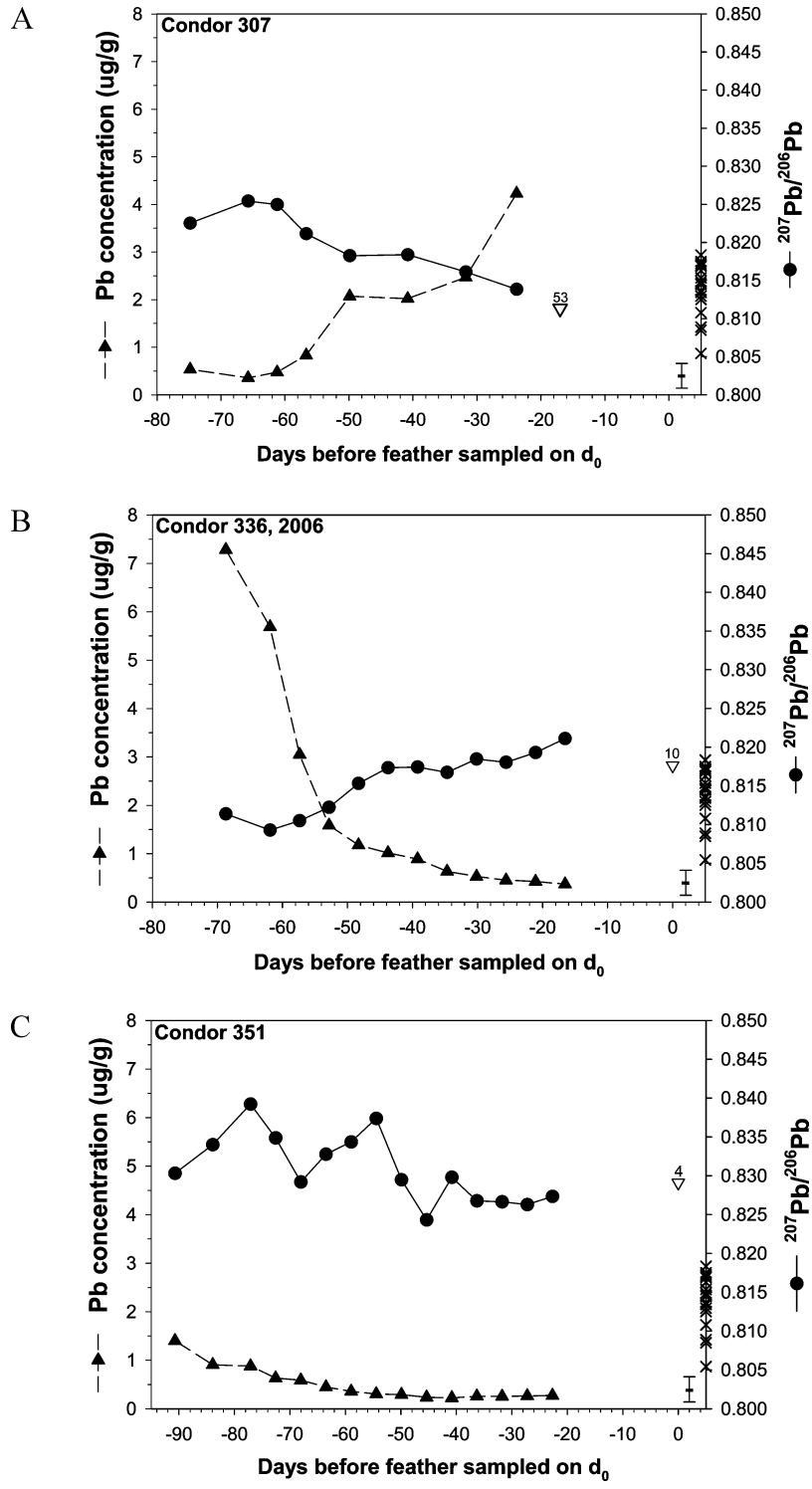

FIGURE 3. Primary feather vane $^{207}Pb/^{206}Pb$ ratios (●) and lead concentrations (▲) versus days before d_0, the date the feather was sampled for condors 307 (A), 336 (B, 14th of December, 2006, collection), and 351 (C). Also shown are $^{207}Pb/^{206}Pb$ ratios in blood (▽) collected at the time of feather vane samples with the exception of condor 307 where feather vane was collected 17 days prior to blood; numbers adjacent to symbols indicate blood lead concentration (μg/dL); (×; along the right *y*-axis) $^{207}Pb/^{206}Pb$ ratios measured in California ammunition (data from Church et al. (*4*)). The ICP-MS measurement error is shown in the lower right (—) ± 2 SD.

measurement error (Figure 3C); one explanation is that it may reflect isolated (separated by multiple days) feeding events on prey items with low lead concentrations but different lead isotopic signatures. However, further study is needed to investigate this possible explanation.

Temporal Relationship between Blood and Feather Lead. As opposed to a blood sample, feather vane samples collected from live birds may not reflect a bird's current lead level. This is because feather vane samples can only be collected from live condors once the newly grown vane emerges from the calamus sheath, typically at a distance of ~15 cm from the feather follicle. As a result, the most proximal (i.e., recently grown) feather vane sample that is available for collection from a live bird would reflect lead in the bird's blood ~10−20 days beforehand. In light of this, the analyses of both feather vane and concurrently collected blood samples provide a powerful means to assess both recent and prior lead exposures in condors. For fully intact growing

feathers that are collected postmortem, such as those from condors 336 and 238 (Figure 2A and B), the full feather was able to be analyzed and thus the most recent feather (rachis) growth reflects the bird's current exposure.

In conclusion, we illustrate how feathers can be used for long-term monitoring of lead exposure and as a forensic tool to reconstruct lead poisoning in California Condors. In cases where two or more growing primary feathers are sampled over a year, ≥60% of a bird's annual lead exposure history may be captured, compared to only ∼10% of an annual exposure history reflected in one or two blood samples collected over the same time period. Although past studies have examined feathers to compare and contrast lead exposure within (*13, 27, 28*) and between (*11, 29, 30*) species, these studies examined the entire feather as one sample and do not provide a time series of exposure as we describe here with sequential feather sampling and analysis. Given that the California Condor is a critically endangered species, we suggest that feather lead concentrations can help estimate annual lead exposure risk in the context of assessing population health. Additionally, we illustrate how feather lead isotopic composition may provide evidence for exposure to lead-based ammunition—information that is important for governments currently reviewing ammunition regulation (*31*). Although we focus solely on lead exposure and California Condors, the use of sequential feather sampling to reconstruct exposure history is a robust approach that may be used to evaluate exposure to other environmental contaminants and in other avian species.

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Supporting Information Available

Supporting information SI 1−4, Tables S1−S3, and Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

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