



Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis



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ABSTRACT

Lead poisoning is preventing the recovery of the critically endangered California condor (*Gymnogyps californianus*) and lead isotope analyses have demonstrated that ingestion of spent lead ammunition is the principal source of lead poisoning in condors. Over an 8 month period in 2009, three lead-poisoned condors were independently presented with birdshot embedded in their tissues, evidencing they had been shot. No information connecting these illegal shooting events existed and the timing of the shooting(s) was unknown. Using lead concentration and stable lead isotope analyses of feathers, blood, and recovered birdshot, we observed that: i) lead isotope ratios of embedded shot from all three birds were measurably indistinguishable from each other, suggesting a common source; ii) lead exposure histories re-constructed from feather analysis suggested that the shooting(s) occurred within the same timeframe; and iii) two of the three condors were lead poisoned from a lead source isotopically indistinguishable from the embedded birdshot, implicating ingestion of this type of birdshot as the source of poisoning. One of the condors was subsequently lead poisoned the following year from ingestion of a lead buckshot (blood lead 556 $\mu\text{g}/\text{dL}$), illustrating that ingested shot possess a substantially greater lead poisoning risk compared to embedded shot retained in tissue (blood lead $\sim 20 \mu\text{g}/\text{dL}$). To our knowledge, this is the first study to use lead isotopes as a tool to retrospectively link wildlife shooting events.

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1. Introduction

Lead isotope analysis is an established technique to identify sources and pathways of lead exposure to humans (Gwiazda and Smith, 2000; Smith et al., 1996; Sturges and Barrie, 1987) and wildlife (Finkelstein et al., 2003; Outridge et al., 1997; Scheuhammer and Templeton, 1998; Smith et al., 1992). We have used lead isotopes to help establish that spent lead ammunition is the principal source of lead poisoning to free-flying California condors (*Gymnogyps californianus*) in California (Church et al., 2006; Finkelstein et al., 2012). We have also shown that analysis of sequential feather segments can be used to reconstruct a condor's lead exposure history over the 2–4 month timeframe of feather growth (Finkelstein et al., 2010). Here we build upon this work to examine three cases of illegal shooting(s) of the critically endangered California condor.

The California condor approached extinction in 1982 with a world population of only 22 individuals (Snyder and Snyder, 2000). Since then, the release of captive-reared birds into the wild in combination with management by government and non-profit agencies have led to a steady increase in the condor population (Walters et al., 2010). As of 30 April 2014 there were 433 California condors, approximately half of which were free flying and associated with release programs in California (134 birds) and Arizona (75 birds), USA, as well as Baja California MX (29 birds) (USFWS unpublished data).

California condors are routinely lead poisoned from feeding on carcasses contaminated by spent lead ammunition and require ongoing intensive management and supportive care to prevent lead-related mortalities (Church et al., 2006; Finkelstein et al., 2012; Parish et al., 2009; Walters et al., 2010). In addition to deaths from lead poisoning, condors face other threats such as morbidity/mortality from gunshot; since 1992 four condors have died as a result of gunshot wounds (Rideout et al., 2012). The shooting of nongame wildlife is illegal and punishable by fines of up to \$2000 (California Rules of Court, 2011). The shooting of a federally

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recognized endangered species triggers an additional violation of federal law punishable by a fine of up to \$50,000 or 1 year imprisonment (U.S. Fish and Wildlife Service, 2003). Enforcement of illegal shooting laws may also receive high priority in cases involving endangered species, as each incidence of injury or death can jeopardize the success of publicly-funded endangered species recovery programs.

All free-ranging condors in California are fit with radio and/or GPS transmitters to monitor their movements on a near daily basis. Condors are recaptured approximately twice per year for health and lead exposure monitoring as well as tag/transponder maintenance, and more frequently if injury or risk of lead poisoning is suspected. Field screening of blood lead levels (LeadCare I or II field measurement kits, Magellan Diagnostics) followed by measurement through an accredited laboratory and archiving of blood samples for possible stable lead isotope analyses are standard procedures. Between March and October 2009 three California condors independently presented with lead poisoning and were transported to the Gottlieb Animal Health and Conservation Center (LA Zoo, California, USA) for clinical management, including chelation therapy. All three birds were identified via radiograph to possess birdshot embedded in their tissues, indicating they had been shot. After the second of the three cases was discovered, efforts were undertaken to identify the person (s) responsible for the shootings, including the offering of a \$40,500 reward for information leading to the arrest and conviction of the perpetrator(s) (Sahagun, 2009). However, as of May 2014, little to no information about the circumstances surrounding the shooting(s) has surfaced and no arrests have been made.

Here we retrospectively investigated these three incidents of illegal California condor shootings using lead concentration and stable lead isotope analysis of condor tissues (e.g. blood and feathers) and recovered embedded and ingested shot. This retrospective case study investigation was possible because of prior establishment of standardized protocols for the routine collection and archiving of blood and feather samples from free-flying condors in California (Appendix A). The preponderance of evidence suggested that the three California condor shootings were related, and possibly resulted from a single shooting event. We also provide evidence that the lead poisoning risk from ingested shot is substantially greater than the poisoning risk from lead shot embedded in tissue.

2. Materials and methods

2.1. Study subjects and sample collections

See also Table A1 for detailed timeline of events and Appendix A for sample collection details.

2.1.1. Illegal shooting event case study

This study presents cases of three California condors (Studbook IDs 286, 375, and 401) who were independently recaptured at trapping sites in central California, found to be lead poisoned with blood lead values of “High” (LeadCare, Magellan Diagnostics), and transported to the LA Zoo for radiographs [Eklin EDR6 Digital Radiograph System (Rapid Start)] and clinical management (e.g. chelation therapy) as per standard procedure. All three birds were discovered to contain multiple embedded birdshot pellets (condor 286 on 4 March 2009 with 10 birdshot pellets, condor 375 on 26 March 2009 with three birdshot pellets, and condor 401 on 30 October 2009 with four birdshot pellets). Based on field observations preceding presentation at the LA Zoo, all three condors were capable of flight and displayed no outward signs of traumatic injury; examinations within the clinic indicated that all pellet entry wounds had healed by the time of radiographic discovery. Radiographic and clinical exams showed that birdshot in condors 375 and 401 was embedded in their soft tissues (muscle, coelomic cavity, gastrointestinal tract, etc.) and not in the joint and/or bone; for 286, the radiographic and clinical exams indicated the birdshot was most likely embedded in soft tissue, yet this assessment was not definitive. Birdshot were recovered surgically (375=one pellet, 401=one pellet) or post-mortem (286=five pellets). At the same time, condor tissues (blood

and feathers) were collected from all three condors, or in the case of 401's feather, marked for future collection once grown-in. Condor 286 died of lead toxicosis on 11 May 2009 (Rideout et al., 2012) and samples of liver, kidney, and bone were collected at necropsy. Condor 401 had additional blood and feather tissue samples collected on 12 April and 27 May 2009.

2.1.2. Condor 401– ingested buckshot

On 21 June 2010 condor 401 again presented with lead poisoning (blood lead value of “High”, LeadCare, Magellan Diagnostics) and was transported to the LA Zoo for treatment where radiographs revealed a buckshot pellet in the bird's gastrointestinal tract; the buckshot was subsequently collected following regurgitation and a second previously identified embedded birdshot pellet was surgically removed from the bird's soft tissue. Tissue samples were collected (blood) or marked for future collection (feather) at the time the bird presented with lead poisoning.

2.2. Sample processing and analysis

Biological and birdshot/buckshot pellet samples were processed and analyzed using established trace metal clean techniques, as described elsewhere (Finkelstein et al., 2003, 2010, 2012; Gwiazda et al., 2005; Smith et al., 1996). For primary feathers, individual sections of feather vane (~2 cm width along rachis axis) were treated as separate samples; each feather section was weighed and then processed under trace metal clean conditions to remove surface contamination by washing sequentially with acetone, ultrapure water, 1% HNO₃ and ultrapure water, as previously reported (Church et al., 2006; Finkelstein et al., 2010). All biological samples (feather, whole blood, liver, kidney, and bone) were processed as described previously (Finkelstein et al., 2003, 2010; Gwiazda et al., 1998; Smith et al., 1996); briefly, samples were digested overnight in 2 mL sub-boiling concentrated HNO₃ in closed Teflon vials, evaporated to dryness, and reconstituted in 1% HNO₃ for analysis. Birdshot/buckshot pellets were individually cleaned and then leached in 1 mL 1% HNO₃ for 30 s for analyses, as previously described (Finkelstein et al., 2012).

Sample lead concentrations and isotope ratios were determined by inductively coupled plasma mass spectrometry (ICP-MS, Finnigan MAT Element magnetic sector), measuring masses of ²⁰⁶Pb, ²⁰⁷Pb, and ²⁰⁸Pb as previously described (Finkelstein et al., 2003; Gwiazda et al., 1998). Added ²⁰⁵Tl was used as an internal standard. The precision of the lead isotope ratio measurements was ~0.10% (2 × the relative standard deviation, 2RSD), based on condor tissue samples analyzed in triplicate within an analytical run. Between-run (i.e. long-term over several years) measurement precision was <0.20% (2RSD), based on repeated measurements of blood and lead ammunition leachate samples. Isotope ratios (²⁰⁷Pb/²⁰⁶Pb) that differed by <0.20% (i.e. the 2RSD of long-term measurement precision) were considered measurably indistinguishable.

3. Results and discussion

3.1. Overview

The discovery of the embedded birdshot in condor 375, 3 weeks after condor 286 similarly presented with embedded birdshot, initiated a high priority analytical assessment of the biological samples associated with these cases (Fig. 1). Within 2 months we determined that the birdshot removed from condors 286 and 375 had lead isotope ratios that were measurably indistinguishable from one another. Lead concentrations and isotopic compositions were then measured in stored blood and feather tissues from these two birds, as well as in samples from condor 401 after the discovery ~8 months later (Oct 2009) of embedded birdshot indicating that this condor had also been shot (Fig. 1, see Table A1 for timeline details). While California condors are monitored on a near daily basis, with many birds being tracked by satellite telemetry (Walters et al., 2010), none of the three birds (condors 286, 375, or 401) were fitted with a satellite transmitter during the timeframe of the presumed shooting(s). Furthermore, the near daily tracking data collected by field biologists did not provide sufficient information about the locations or spatial associations of these birds that could be used to infer the timing or location of the shooting(s).

Based on the preponderance of lead concentration and isotopic composition evidence from blood, feather, and birdshot pellet samples, we propose that all three condors were shot in a common

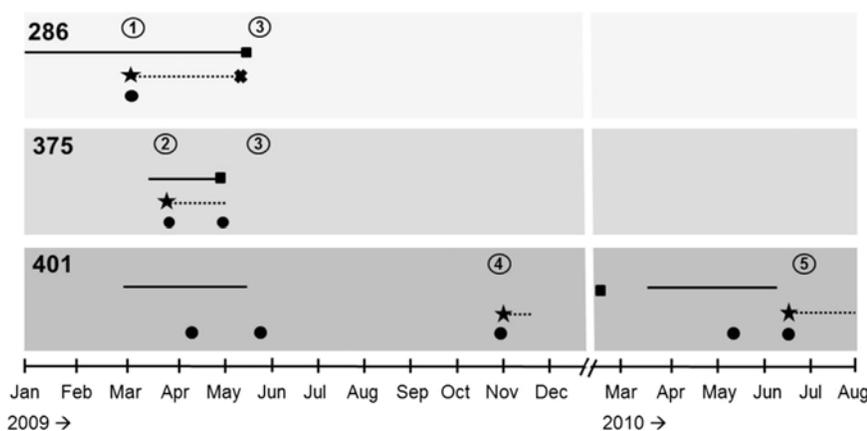


Fig. 1. Timeline of condor feather growth (estimated), sample collection, and analysis for the illegal shooting cases of California condors 286, 375, and 401 (see also Table A1). Event ① 5 March 2009, radiograph of condor 286 revealed birdshot embedded in his tissues. Event ② 26 March 2009, radiograph of condor 375 also showed birdshot embedded in her tissues, triggering high priority collection of feather samples from condors 286 and 375. Event ③ lead isotope analysis determined that birdshot pellets removed from condors 286 and 375 had $^{207}\text{Pb}/^{206}\text{Pb}$ ratios that were measurably indistinguishable from each other. Event ④ 1 November 2009, radiograph of condor 401 revealed birdshot embedded in his tissues, prompting analysis of previously collected feather and blood samples; analysis determined that the pellet removed from condor 401 had a lead isotopic signature that was measurably indistinguishable from the birdshot pellets removed from condors 286 and 375. Event ⑤ 21 June 2010, radiograph revealed condor 401 ingested a radio-opaque object, which after regurgitation and collection was identified as a buckshot pellet. ★ designates when a condor was radiographed, dotted line (—) corresponds to when a condor was hospitalized for clinical management of lead poisoning at the Gottlieb Animal Health and Conservation Center (LA Zoo), • designates a blood sampling event by condor field biologists, straight line (—) corresponds to the estimated timeframe of feather growth, ‘ designates when a feather sample was collected, and x represents a mortality event (condor 286). Blood samples for lead concentration and lead isotope analyses were collected simultaneously with blood samples used for lead screening in the field. Condors were free-flying unless captured for a blood sampling event or hospitalized for chelation therapy.

shooting event. While none of the three birds died from their gunshot wounds, two of the birds (condors 286 and 375) were poisoned by a lead source with a $^{207}\text{Pb}/^{206}\text{Pb}$ ratio that was measurably indistinguishable from the embedded birdshot. Notably, one of these birds (condor 286) died from lead toxicosis on 11 May 2009 (Rideout et al., 2012) as a result of this lead poisoning event.

Condor 401 subsequently presented again with lead poisoning in June 2010 due to ingested buckshot identified via radiograph. Since protocols for the routine collection and storage of condor blood and feather samples had been previously established (see Appendix A), archived tissue samples were available for condor 401 to assess the magnitude of lead exposure from ingested versus tissue-embedded lead shot within the same individual.

3.2. Reconstructed illegal shooting of California condors 286, 375 and 401

3.2.1. Lead isotopic signatures of condor blood and recovered birdshot pellets were measurably indistinguishable from one another

A total of eight embedded birdshot pellets were recovered from condors 286 ($n=5$), 375 ($n=1$), and 401 ($n=2$) during necropsy (condor 286) or treatment for lead poisoning (condors 375 and 401) between April 2009 and June 2010 (Table A1). All eight pellets were similar in appearance and their lead isotopic compositions were measurably indistinguishable from one another (average $^{207}\text{Pb}/^{206}\text{Pb}=0.81887 \pm 0.0005\text{SD}$, $n=8$, Table A2). The range of diameters and weights for the pellets (2.61–2.97 mm and 105–136 mg, respectively) are consistent with either #6 or #7 birdshot. However, since the mass and shape of these pellets have most certainly been altered from being fired and striking the birds, with some of the pellets appearing to have tissue residue on their surface that may affect these measurements, the range in diameter and mass reported above is not unexpected but precludes a more precise classification of the shot.

Condors 286 and 375 presented with lead poisoning (blood lead levels of 155 and 180 $\mu\text{g}/\text{dL}$ on 4 March and 26 March 2009, respectively) when they were identified via radiograph to contain embedded birdshot in their tissues. For 286, the

$^{207}\text{Pb}/^{206}\text{Pb}$ ratio of the blood sample collected at the time he presented with lead poisoning was measurably indistinguishable from the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of the five birdshot pellets recovered from his tissues (blood $^{207}\text{Pb}/^{206}\text{Pb}=0.8194$, birdshot $^{207}\text{Pb}/^{206}\text{Pb}=0.8183\text{--}0.8194$). Condor 375's blood isotope ratio was very similar to, yet measurably different from, the single birdshot pellet recovered from her tissues (blood $^{207}\text{Pb}/^{206}\text{Pb}=0.8225$, recovered birdshot $^{207}\text{Pb}/^{206}\text{Pb}=0.8184$, Table A2). In both cases, the data suggest either that the source of lead poisoning was the embedded birdshot and/or ingestion of a lead source (unrecovered) that was isotopically similar to the embedded birdshot.

Condor 401 on 12 April 2009 was found to have a field blood lead level that was elevated according to Cade (2007) (11 $\mu\text{g}/\text{dL}$, LeadCare, Magellan Diagnostics) but below the threshold indicating clinical treatment (35 $\mu\text{g}/\text{dL}$); thus, condor 401's blood sample was archived per established protocol (Appendix A). Condor 401's archived blood sample was prioritized for analysis after he presented ~8 months later (30 October 2009) with lead poisoning (86 $\mu\text{g}/\text{dL}$, Louisiana Animal Disease Diagnostic Laboratory) and identified via radiograph to contain tissue-embedded birdshot. The $^{207}\text{Pb}/^{206}\text{Pb}$ ratio of condor 401's 12 April 2009 blood sample ($^{207}\text{Pb}/^{206}\text{Pb}$ blood=0.8166, lead concentration 16.6 $\mu\text{g}/\text{dL}$) was measurably indistinguishable from his recovered birdshot (average $^{207}\text{Pb}/^{206}\text{Pb}=0.8187$, $n=2$, Table A2).

3.2.2. Feather lead concentrations and isotopic compositions are consistent with the suggestion that condors 286, 375, and 401 were shot in late January 2009 and support the conclusion that the three condors were exposed to a lead source measurably indistinguishable from the embedded pellets.

We have previously established that feathers can be used to reconstruct a condor's lead exposure history over the 3–4 month timeframe of feather growth, and that the relationship between blood lead ($\mu\text{g}/\text{dL}$) and feather lead ($\mu\text{g}/\text{g}$) concentrations (i.e. blood lead:feather lead ratio) is ~19:1 (Finkelstein et al., 2010). Here we used this approach to reconstruct the lead exposure histories of condors 286, 375 and 401. Fortuitously, these three condors had primary feathers that were growing over the period

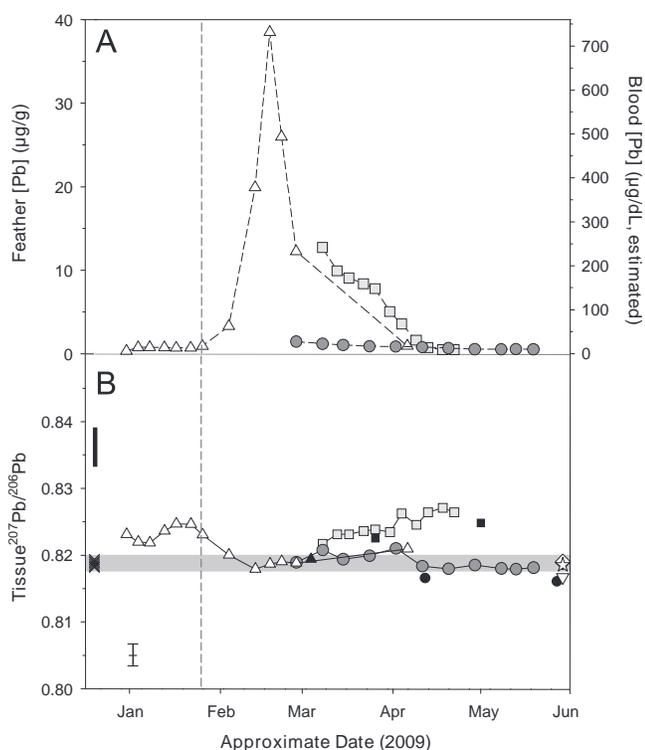


Fig. 2. Panel A) Condor 286 (\triangle), 375 (\square) and 401 (\bullet) feather lead concentrations (left axis) versus estimated calendar date. Estimated blood lead concentrations (right y-axis) calculated from measured feather lead concentrations using a blood ($\mu\text{g}/\text{dL}$):feather ($\mu\text{g}/\text{g}$) lead concentration relationship of 19:1 (Finkelstein et al., 2010). Panel B) Feather $^{207}\text{Pb}/^{206}\text{Pb}$ ratios for condors 286 (\triangle), 375 (\square), and 401 (\bullet) versus estimated calendar date. Blood $^{207}\text{Pb}/^{206}\text{Pb}$ ratios for condors 286 (\blacktriangle), 375 (\square), and 401 (\bullet) (plotted on collection date) are similar to the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of embedded birdshot (X, $n=8$, left axis) recovered from all three birds. Black vertical bar on y-axis represents the average \pm 2RSE (residual standard error) background $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in pre-release condors in California (0.8362 ± 0.0028 , $n=22$) (Finkelstein et al., 2012). Horizontal shaded bar represents the average \pm 2RSD of the recovered embedded birdshot pellets (0.8188 ± 0.0012 , $n=8$). Also shown are $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of tissues from condor 286 (\diamond liver, ∇ kidney, \star bone). The error bar in lower left reflects the $^{207}\text{Pb}/^{206}\text{Pb}$ measurement error (i.e. 2RSD, see Materials and methods). Estimated date of shooting event in late January 2009 (dotted line in both panels) based on condor 286's feather lead profile inflection point corresponding to an increase in feather lead concentration and a decrease in the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios. Calendar date estimated from feather length using a primary feather growth rate of 4.4 mm/day (Finkelstein et al., 2010) (Appendix B).

that blood samples were collected and near the time of the suspected shooting(s) (Figs. 1 and 2).

3.2.2.1. Feather lead concentrations. The feather lead concentration profile from condor 286 indicated that the bird was initially lead exposed at the end of January 2009 with lead concentrations reaching a peak of 39 $\mu\text{g}/\text{g}$ by mid-February (equivalent to $\sim 730 \mu\text{g}/\text{dL}$ estimated blood lead). For condor 375, the feather did not start growing until after this bird's peak lead exposure had occurred, as evidenced by a feather lead concentration profile that is clearly decreasing from a prior acute exposure event; that prior exposure event appears to be of a similar magnitude to condor 286's peak exposure (Fig. 2A). Similarly, condor 401's feather also did not start growing until after the estimated time of 286's lead poisoning (end of January 2009) with the lead concentration profile indicating that condor 401 did not experience an acute lead poisoning event as evidenced in condors 286 and 375 (Fig. 2A). Rather, condor 401's feather lead concentration profile illustrated that he was moderately lead exposed at the time the

feather started growing with a feather lead concentration of 1.45 $\mu\text{g}/\text{g}$ (equivalent to 27 $\mu\text{g}/\text{dL}$ estimated blood lead).

Given that the feathers from condors 286 and 375 illustrated that these birds were exposed to an acute lead poisoning event (Fig. 2A), and that the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of their blood samples at the time they presented with lead poisoning were very similar to their embedded shot (Fig. 2B), we propose that the most plausible cause of lead poisoning in these two birds was from ingestion of a lead source with an isotopic signature of their embedded pellets. This might have occurred either through feeding on a carcass that was contaminated with birdshot identical to the bird's tissue-embedded pellets, or from ingestion of tissue-embedded pellet (s) that they preened from their wounds. In contrast, we propose that condor 401, with a moderately elevated blood lead level, was exposed only from the tissue-embedded birdshot—a suggestion supported by a study in humans that reported elevated blood lead levels (range 7–50 $\mu\text{g}/\text{dL}$) in patients with embedded lead shrapnel in their tissues (Farrell et al., 1999). Although, we could not exclude the possibility that 401 was moderately lead exposed from a single ingestion and rapid regurgitation of a birdshot measurably indistinguishable from the type found embedded in the condors.

3.2.2.2. Feather lead isotopic compositions. Feather lead isotopic compositions also support the conclusion that the birds were exposed to a lead source measurably indistinguishable from the embedded birdshot pellets (Fig. 2B), corroborating the findings from the blood lead isotope results. Corresponding to a change in the source of lead exposure, the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in condor 286's feather started to decline at the same time the lead concentrations started to increase (i.e. end of January 2009, Fig. 2). Notably, the lead isotope ratio in 286's feather segment possessing the highest (peak) lead concentration ($^{207}\text{Pb}/^{206}\text{Pb}=0.8187$, 38.5 $\mu\text{g}/\text{g}$) is measurably indistinguishable from the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of the embedded birdshot and blood collected at the time he presented with lead poisoning (Fig. 2B). As stated above, condor 286 subsequently died from lead toxicosis as a result of this lead poisoning event. The $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in condor 286's liver, kidney, and tibiotarsus samples collected post-mortem were also measurably indistinguishable from the blood, feather, and embedded birdshot collected over the exposure period (Fig. 2B), further evidencing that 286 was severely lead poisoned by a lead source with $^{207}\text{Pb}/^{206}\text{Pb}$ ratios measurably indistinguishable from the embedded shot.

For condor 375, the first-to-grow (i.e. oldest) feather segment with the highest lead concentration (12.7 $\mu\text{g}/\text{g}$, corresponding to an estimated blood lead of 240 $\mu\text{g}/\text{dL}$) had a $^{207}\text{Pb}/^{206}\text{Pb}$ ratio that was measurably indistinguishable from the recovered birdshot (Fig. 2). This supports the conclusion that prior to blood sampling on 26 March 2009, condor 375 also had a blood lead isotopic composition that was measurably indistinguishable from the recovered birdshot. As expected, the two blood samples taken during the period of feather growth, one at the time the bird presented with lead poisoning on 26 March 2009 (blood lead 180 $\mu\text{g}/\text{dL}$) and the other taken prior to the bird's re-release after chelation treatment for lead poisoning (1 May 2009, blood lead 34.7 $\mu\text{g}/\text{dL}$), had $^{207}\text{Pb}/^{206}\text{Pb}$ ratios consistent with the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in feather segments growing at the time of blood collection (Fig. 2B).

Condor 401 did not experience an acute lead poisoning event during the timeframe of feather growth (Fig. 2A); the feather lead concentration profile indicated that the bird had experienced a moderate lead exposure event (estimated blood lead of 27 $\mu\text{g}/\text{dL}$, Fig. 2A) that we attributed to lead from the tissue-embedded shot, as noted previously. While condor 401 was not severely lead

poisoned, the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of both the feather and blood were measurably indistinguishable from the recovered birdshot pellets, indicating that condor 401's tissue lead isotopic signature was heavily influenced by this moderate lead exposure event (Fig. 2B).

3.2.3. Commonalities between condor shooting events and study limitations

Given the preponderance of evidence presented above (see also Table 1), we conclude that the three cases of condor shootings are linked, and possibly from a single shooting event. This conclusion is supported by: 1) the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of recovered birdshot and blood (condors 286 and 401) and feather (condors 286, 375, and 401) samples are measurably indistinguishable from one another, and 2) the feather lead concentration and isotopic composition profiles are consistent with the suggestion that condors 286, 375 and 401 were exposed to an elevated lead source within the same timeframe.

While we consider this the most likely scenario, this conclusion may be qualified by several limitations of the study. First, unlike the feather from condor 286, the feathers collected from condors 375 and 401 did not start growing until after the estimated time of exposure, and so their feather lead concentration and isotopic composition profiles did not capture the peak exposure event. Nonetheless, the feather lead profiles of condors 375 and 401 are consistent with, and in fact cannot exclude, a lead exposure event in late January 2009 to a lead source common across all three cases. Second, the fact that the lead isotope signatures in the condor tissues and the recovered birdshot were measurably indistinguishable from one another did not by itself prove that the lead poisonings in all three birds arose from a single source of birdshot. There has not been a systematic evaluation of the isotopic variability of birdshot within a single shotshell cartridge, among cartridges within a box, or among boxes within or across manufacturers for birdshot ammunition available within the central California range. However, we have evaluated the isotopic composition of lead bullet and shotgun ammunition from California ($n=73$) (Finkelstein et al., 2012), and only five ammunition samples ($\sim 7\%$) have lead isotopic compositions that fell within the range of the recovered embedded birdshot from the three condors (Fig. 4). Further, Scheuhammer and Templeton (1998) reported

that “The within-cartridge variability in the $^{206}\text{Pb}:^{207}\text{Pb}$ ratios for pellets taken from the same shotshell cartridge was very low ($\text{CV} < 0.5\%$) and this was also true for pellets from different cartridges from the same box ($\text{CV} < 0.3\%$). Although there was considerable variability in the $^{206}\text{Pb}:^{207}\text{Pb}$ ratios between different brands of shot...”. Thus, in total, the evidence presented suggests that the birds were shot by a common source at the same time, although we could not exclude the possibility that three independent shooting events occurred within the same timeframe with shot that was measurably indistinguishable from each other.

3.3. Ingested lead shot results in higher blood lead compared to embedded lead shot

Condor 401 presented again with clinical lead poisoning in June 2010 (blood lead 556 $\mu\text{g}/\text{dL}$), when a radiograph showed an ingested buckshot within the bird's gastrointestinal tract as well as the three remaining embedded birdshot pellets that had been discovered the previous year (Fig. A1). The $^{207}\text{Pb}/^{206}\text{Pb}$ ratio (0.8130) of condor 401's blood collected at the time of acute lead poisoning on 21 June 2010 was measurably indistinguishable from the lead isotope ratio of the recovered ingested buckshot ($^{207}\text{Pb}/^{206}\text{Pb}=0.8122$) and most recently grown feather section ($^{207}\text{Pb}/^{206}\text{Pb}=0.8142$) coinciding with the peak of exposure (Fig. 3). Of significance is that the isotopic composition of the buckshot is measurably different from the recovered embedded birdshot (average birdshot $^{207}\text{Pb}/^{206}\text{Pb}=0.8188$, Fig. 3B, Table A2).

Condor 401's feather lead concentration profile captured the May/June 2010 severe lead poisoning event that is attributed to the ingested buckshot, with a peak feather lead concentration of 53.7 $\mu\text{g}/\text{g}$, corresponding to an estimated blood lead of 1000 $\mu\text{g}/\text{dL}$ (Fig. 3A). Condor 401's feather completed growing soon after this acute exposure event and did not reflect the decline in body lead levels associated with clinical chelation treatment in June 2010. Noteworthy is that condor 401's feather also showed evidence of an additional lead exposure event, likely several weeks to months before the feather started growing in mid-March 2010 (Fig. 3). While the peak lead exposure from this event occurred prior to feather growth, the oldest (i.e. first-to-grow) feather segment had a lead concentration (3.36 $\mu\text{g}/\text{g}$) that corresponded to an estimated blood lead level $> 60 \mu\text{g}/\text{dL}$, which is above the blood lead

Table 1
Summary of lead concentration and isotopic composition evidence from blood, feather, and recovered birdshot samples supporting commonalities between shooting events of condors 286, 375, and 401 (see also Fig. 2). The evidence is consistent with the conclusion that the three cases of condor shootings are linked, possibly through the same shooting event.

Evidence	Condor			Interpretation
	286	375	401	
Lead $^{207}\text{Pb}/^{206}\text{Pb}$ ratios of recovered birdshot measurably indistinguishable	Yes, $n=5$ birdshot	Yes, $n=1$ birdshot	Yes, $n=2$ birdshot	Condors were shot in the same event and/or by the same individual ^a
Condors had elevated blood lead concentrations with an isotopic composition measurably indistinguishable from the embedded birdshot	Yes, 155 $\mu\text{g}/\text{dL}$ blood lead	Yes, 180 $\mu\text{g}/\text{dL}$ blood lead	Yes, 17 $\mu\text{g}/\text{dL}$ blood lead	Condors lead exposed by either ingested (condors 286, 375) or embedded (condor 401) lead birdshot
Feather lead concentration profiles consistent with a distinct lead exposure event occurring in late January 2009	Peak exposure event fully captured; estimated $\sim 730 \mu\text{g}/\text{dL}$ peak blood lead	Peak exposure event not fully captured; estimated $> 240 \mu\text{g}/\text{dL}$ blood lead	Peak exposure event not fully captured; $\sim 27 \mu\text{g}/\text{dL}$ highest blood lead	Feather lead concentration are consistent with an acute (condors 286, 375) or moderate (condor 401) exposure event that occurred in late January
Feather $^{207}\text{Pb}/^{206}\text{Pb}$ ratio profiles show exposures were to a lead source measurably indistinguishable isotopically from the recovered embedded birdshot	$^{207}\text{Pb}/^{206}\text{Pb}$ ratio of peak exposure measurably indistinguishable from birdshot	$^{207}\text{Pb}/^{206}\text{Pb}$ ratio in first to grow feather segment measurably indistinguishable from birdshot	$^{207}\text{Pb}/^{206}\text{Pb}$ ratios of feather segments measurably indistinguishable from birdshot	Feather $^{207}\text{Pb}/^{206}\text{Pb}$ ratio profiles are consistent with an acute (condors 286, 375) or moderate (condor 401) exposure event that occurred in late January from either ingested (condors 286, 375) or embedded (condor 401) birdshot

^a Existing data suggest that lead isotope ratios in ammunition vary between type and manufactures, which decreases the likelihood that shot from different events would be measurably indistinguishable from each other (Fig. 4) (Finkelstein et al., 2012; Scheuhammer and Templeton, 1998).

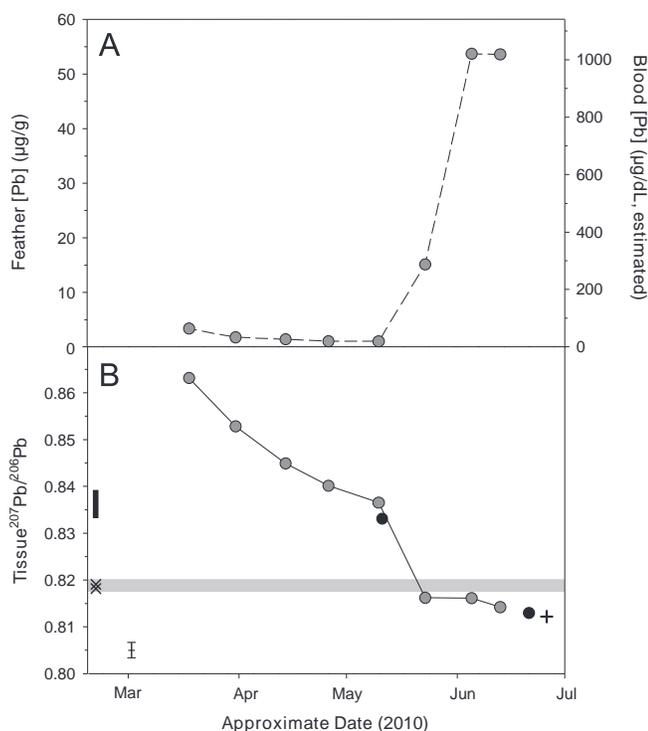


Fig. 3. Panel A) Condor 401 feather lead concentration (left axis) (—●) versus estimated calendar date. Blood lead concentrations (estimated, right y-axis) calculated from measured feather lead concentrations using a blood (µg/dL):feather (µg/g) lead concentration relationship of 19:1 (Finkelstein et al., 2010). Panel B) Condor 401 feather ²⁰⁷Pb/²⁰⁶Pb ratios (—●) versus estimated calendar date. Also shown are ²⁰⁷Pb/²⁰⁶Pb ratios of blood samples (●), embedded birdshot pellets (X, $n=2$, left axis) and ingested buckshot (+) recovered from condor 401 during treatment for lead poisoning (plotted on collection date). Noteworthy is that 401's oldest (i.e. first-to-grow) feather segment had a ²⁰⁷Pb/²⁰⁶Pb ratio of 0.8631 and a lead concentration of 3.36 µg/g (estimated blood lead level ~60 µg/dL); the lead concentration and isotope ratio profiles evidenced that this bird was recovering from a prior lead exposure event to a source with a ²⁰⁷Pb/²⁰⁶Pb signature Z 0.86. Beginning in the middle of May 2010, approximately 2 months after the feather started to grow, the feather ²⁰⁷Pb/²⁰⁶Pb ratios measurably decline (from 0.8365 to 0.8142), while the feather lead concentrations increase until the last feather segment (from ~1 to 53.6 µg/g); the ²⁰⁷Pb/²⁰⁶Pb ratio in the final, newest-to-grow segment is measurably indistinguishable from the isotope ratios of the blood and ingested buckshot. Black vertical bar on y-axis represents the average \pm 2RSE background lead ²⁰⁷Pb/²⁰⁶Pb ratios in pre-release condors in California (²⁰⁷Pb/²⁰⁶Pb = 0.8362 \pm 0.0028, $n=22$) (Finkelstein et al., 2012). Horizontal shaded bar represents the average ²⁰⁷Pb/²⁰⁶Pb value (\pm 2RSD) of the recovered embedded birdshot pellets (0.8188 \pm 0.0012, $n=8$). The error bar in lower left reflects the ²⁰⁷Pb/²⁰⁶Pb measurement error (i.e. 2RSD, see methods). Calendar date estimated from feather length using a primary feather growth rate of 4.4 mm/day (Finkelstein et al., 2010) (Appendix B).

threshold indicating clinical chelation for condors (~35 µg/dL). Therefore, blood and feather analyses showed that condor 401 experienced at least four lead poisoning events (blood lead > 35 µg/dL) throughout the ~1.5 year study period, with the bird being hospitalized for clinical treatment of lead poisoning three times (see also Table A1).

A comparison of the resultant blood lead levels from ingested versus embedded lead shot in condor 401 demonstrates that ingested lead shot produces substantially higher (~30-fold) blood lead levels than tissue-embedded lead shot (i.e. 556 versus 16.6 µg/dL). While many factors can influence the dissolution of lead from embedded shot into surrounding tissues, such as the number, size, and surface area of birdshot versus buckshot pellets acquired by condor 401, the comparison of blood lead values clearly indicates that lead shot ingestion possesses a substantially greater exposure risk compared to embedded shot. This result further supports our conclusion that the severe lead poisoning

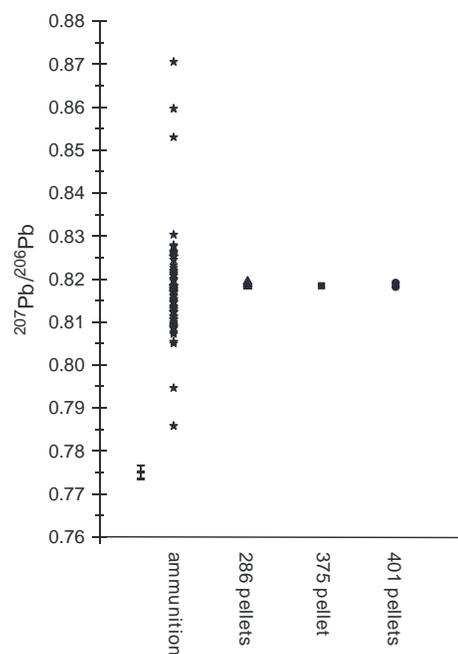


Fig. 4. ²⁰⁷Pb/²⁰⁶Pb ratios of the embedded birdshot pellets recovered from condors 286 (▲, range=0.8183–0.8194, $n=5$), 375 (◻, 0.8184, $n=1$), 401 (●, 0.8182, 0.8191, $n=2$), and ammunition samples from California [★, range=0.7858–0.8706, $n=73$, from Finkelstein et al. (2012)]. Only five out of 73 ammunition samples (~7%) fall within the range of the recovered embedded birdshot pellets, supporting our suggestion that the recovered birdshot pellets originated from a common shooting event. The error bar in lower left reflects the ²⁰⁷Pb/²⁰⁶Pb measurement error (i.e. 2RSD, see Materials and methods).

cases of condors 286 and 375 (blood leads 155 and 180 µg/dL, respectively) are likely due to ingestion of birdshot and not the embedded pellets in their tissues.

4. Conclusion

Our study illustrates the utility of lead isotope analysis of blood and feather tissues to inform circumstances surrounding the illegal shooting of the endangered California condor. The case of 401 also supports our prior findings that feathers are an important tool to understand a condor's true lead poisoning risk (Finkelstein et al., 2010, 2012). Blood and feather analyses of condor 401 identified four lead poisoning events during the 1.5 year study period, a finding underscoring the epidemic lead poisoning rates observed in condors in California (Finkelstein et al., 2012). Notably, use of lead-based ammunition within the condor range was restricted in California in 2008 (Ridley-Tree Condor Preservation Act, 2007), prior to the estimated timeframe of the shooting(s) and 401's four identified lead poisoning events. However, this restriction did not include lead birdshot, the type of shot found embedded in the condors, and condor lead exposure rates statewide did not decline as a result of the lead ammunition restriction (Finkelstein et al., 2012). At the national level, restrictions on the use of lead shot for hunting waterfowl in the United States are believed responsible for the reduction of lead poisoning rates in many wildlife species, including ducks (Anderson et al., 2000; Samuel and Bowers, 2000), and in Spain restrictions on the use of lead shot have resulted in a decline in wildlife exposure rates and lower lead levels in game meat (Mateo et al., 2014).

Others have used lead isotope analysis in homicide investigations (Gulson et al., 2002; Stupian et al., 2001), yet to our knowledge, we provide the first use of this approach to retrospectively examine the illegal shooting of an endangered species. Our results

suggest that lead isotope analysis of tissues and recovered ammunition can be utilized for other wildlife species to aid in the identification of potential commonalities between shooting events. Wildlife rehabilitation centers report gunshot injuries to be responsible for as much as 10% of raptor species morbidity and mortality (Deem et al., 1998; Molina-Lopez et al., 2011); thus additional information to understand the circumstances surrounding shooting-related injuries may be beneficial for wildlife conservation, law enforcement, and management.

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Society, and The Wildlife Disease Laboratories at the Institute for Conservation Research San Diego Zoo Global staff for their support of this work. We also thank the constructive comments provided by three anonymous reviewers.

Appendix A

Sample collection protocol

As part of an ongoing collaborative effort to aid in the management and recovery of the California condor, a standardized sample collection protocol for all free-flying condors in California has been established. Typically, whole blood and feather vane samples are collected by field biologists and transferred to the trace metal laboratory at the University of California, Santa Cruz (UCSC) for lead analysis, as follows:

- 1) During routine health monitoring of free-flying California condors (typically in the spring and fall), a whole blood sample (1–3 mL) from the tibiotarsal vein is collected into low-lead Vacutainers with EDTA anti-coagulant (Fisher Scientific, Pittsburgh, PA) using a 19 or 21-gauge catheter, as previously described (Church et al., 2006; Finkelstein et al., 2010). Whole blood samples are stored at $-20\text{ }^{\circ}\text{C}$ until analysis.
- 2) When condors are handled for blood sampling, birds are examined for growing primary feathers. Growing feathers are measured (length, follicle to tip) and marked to facilitate identification for potential future collection, typically by cutting a 3–5 mm notch in the leading edge of the primary feather vane. Upon recapture for a subsequent health check, previously

Table A1

Timeline of case study-related events for condors 286, 375, and 401 from January 2009 through May 2011. Sample collection was performed by Ventana Wildlife Society (VWS), Pinnacles National Park (PNP), the Gottlieb Animal Health and Conservation Center (LAZ), and The Wildlife Disease Laboratories at the Institute for Conservation Research San Diego Zoo Global (SDZ). Field blood lead levels were measured using a LeadCare I or II field measurement kit (Magellan Diagnostics), while additional samples were collected simultaneously for more precise measurements of blood lead concentrations at the University of California, Santa Cruz (UCSC) unless otherwise noted.

Date	Condor ID	Event/action
January 2009	286, 375, 401	Estimated date of shooting event(s)
28 January 2009	286	Behavioral change noted by PNP staff biologists
4 March 2009	286	Trapped at VWS field site due to observed behavioral change, field blood lead level "High", UCSC blood lead level 155 $\mu\text{g}/\text{dL}$
5 March 2009	286	Transported to LAZ for treatment, 10 embedded birdshot pellets identified via radiograph
26 March 2009	375	Trapped by VWS for routine health check, field blood lead level "High", UCSC blood lead level 180 $\mu\text{g}/\text{dL}$, transported to LAZ for treatment, three embedded birdshot pellets identified via radiograph
12 April 2009	401	Trapped by PNP for routine health check, field blood lead level 11 $\mu\text{g}/\text{dL}$, UCSC blood lead level 16.6 $\mu\text{g}/\text{dL}$, growing right primary feather #3 (RP3) measured and marked. Bird re-released to wild
20 April 2009	375	One birdshot pellet surgically removed
1 May 2009	375	Re-released to wild by VWS post-treatment. Prior to release, blood and growing feather collected by VWS, UCSC blood lead level 34.7 $\mu\text{g}/\text{dL}$
11 May 2009	286	Died of lead toxicosis, five embedded birdshot pellets, growing feather, bone, kidney, and liver samples collected by necropsy staff at the SDZ.
27 May 2009	401	Trapped by PNP for routine health check, field blood lead level 13 $\mu\text{g}/\text{dL}$, UCSC blood lead level 18.0 $\mu\text{g}/\text{dL}$, growing feather (RP3) collected. Re-released to wild
30 October 2009	401	Trapped by VWS for routine health check, field blood lead level "High", Louisiana Animal Disease Diagnostics Laboratory blood lead level 86 $\mu\text{g}/\text{dL}$
1 November 2009	401	Transported to LAZ for treatment, four embedded birdshot pellets identified via radiograph
6 November 2009	401	One birdshot pellet surgically removed
19 November 2009	401	Re-released to wild by VWS post-treatment
11 May 2010	401	Trapped by PNP for routine health check, field blood lead level 22 $\mu\text{g}/\text{dL}$, UCSC blood lead level 25.7 $\mu\text{g}/\text{dL}$, growing feather (RP8) measured and notched
21 June 2010	401	Trapped by VWS, field blood lead level "High", UCSC blood lead level 556 $\mu\text{g}/\text{dL}$, transported to LAZ for treatment. Three embedded birdshot pellets in wing and one buckshot shot pellet in digestive tract identified via radiograph (Fig. A1)
22 June 2010	401	One birdshot pellet surgically removed
26 June 2010	401	Ingested buckshot pellet collected from regurgitated casting by LAZ staff
8 September 2010	401	Transferred to PNP and monitored in captivity
6 October 2010	401	Re-released to wild by PNP
18 May 2011	401	Trapped by PNP for routine health check, field blood lead level "High," fully grown feather (RP8) collected
19 May 2011	401	Transported to LAZ for treatment
7 June 2011	401	Re-released to wild by PNP post-treatment

Table A2

Sample collection information, lead concentrations, $^{207}\text{Pb}/^{206}\text{Pb}$ ratios, and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios of tissues collected from condors 286, 375, and 401. Also shown are the $^{207}\text{Pb}/^{206}\text{Pb}$ ratios and $^{208}\text{Pb}/^{206}\text{Pb}$ ratios of the recovered birdshot and buckshot.

Condor ID #	Sample	Sample collection date	Feather segment # (segment length) ^a	[Pb] ^b	$^{207}\text{Pb}/^{206}\text{Pb}$	$^{208}\text{Pb}/^{206}\text{Pb}$
286	Birdshot	5/17/2009	—	—	0.8187	2.0178
286	Birdshot	5/17/2009	—	—	0.8183	2.0183
286	Birdshot	5/17/2009	—	—	0.8194	2.0203
286	Birdshot	5/17/2009	—	—	0.8194	2.0191
286	Birdshot	5/17/2009	—	—	0.8189	2.0179
286	Blood	3/4/2009	—	155	0.8194	2.0150
286	Liver	5/11/2009	—	4.07	0.8190	2.0129
286	Kidney	5/11/2009	—	1.72	0.8167	2.0158
286	Bone	5/11/2009	—	21.0	0.8185	2.0164
286	Feather	Post-mortem	1 (2.0)	0.33	0.8231	2.0327
286	Feather	Post-mortem	3 (2.0)	0.74	0.8220	2.0299
286	Feather	Post-mortem	4 (2.0)	0.76	0.8219	2.0233
286	Feather	Post-mortem	5 (2.0)	0.74	0.8236	2.0320
286	Feather	Post-mortem	6 (2.0)	0.70	0.8247	2.0316
286	Feather	Post-mortem	7 (2.0)	0.69	0.8247	2.0355
286	Feather	Post-mortem	8 (2.0)	0.92	0.8231	2.0317
286	Feather	Post-mortem	9 (2.0)	3.30	0.8200	2.0252
286	Feather	Post-mortem	11 (2.0)	19.9	0.8179	2.0205
286	Feather	Post-mortem	13 (2.0)	38.5	0.8187	2.0242
286	Feather	Post-mortem	14 (2.0)	26.0	0.8191	2.0180
286	Feather	Post-mortem	15 (2.0)	12.3	0.8188	2.0217
286	Feather	Post-mortem	16 (2.5)	0.93	0.8210	2.0203
375	Birdshot	4/20/2009	—	—	0.8184	2.0167
375	Blood	3/26/2009	—	180	0.8225	2.0178
375	Blood	5/1/2009	—	34.7	0.8248	2.0229
375	Feather	5/1/2009	1 (2.0)	12.7	0.8217	2.0167
375	Feather	5/1/2009	2 (2.0)	9.93	0.8231	2.0213
375	Feather	5/1/2009	3 (2.0)	9.07	0.8231	2.0206
375	Feather	5/1/2009	4 (2.0)	8.38	0.8236	2.0225
375	Feather	5/1/2009	5 (2.0)	7.78	0.8238	2.0244
375	Feather	5/1/2009	6 (2.0)	5.04	0.8235	2.0235
375	Feather	5/1/2009	7 (2.0)	3.57	0.8262	2.0270
375	Feather	5/1/2009	8 (2.0)	1.63	0.8246	2.0251
375	Feather	5/1/2009	9 (2.0)	0.73	0.8264	2.0272
375	Feather	5/1/2009	10 (2.0)	0.49	0.8271	2.0239
375	Feather	5/1/2009	11 (1.4)	0.48	0.8264	2.0242
401	Birdshot	11/6/2009	—	—	0.8191	2.0205
401	Birdshot	6/22/2010	—	—	0.8182	2.0185
401	Buckshot	6/26/2010	—	—	0.8122	1.9982
401	Blood	4/12/2009	—	16.6	0.8166	2.0101
401	Blood	5/27/2009	—	18.0	0.8161	2.0124
401	Blood	5/11/2010	—	25.7	0.8331	2.0470
401	Blood	6/21/2010	—	556	0.8130	2.0049
401	Feather	4/12/2009	1 (3.8)	1.45	0.8189	2.0188
401	Feather	5/27/2009	2+3 (4.0)	1.18	0.8208	2.0236
401	Feather	5/27/2009	4 (2.0)	1.05	0.8194	2.0177
401	Feather	5/27/2009	5 (2.0)	0.89	0.8199	2.0170
401	Feather	5/27/2009	7 (2.0)	0.85	0.8210	2.0211
401	Feather	5/27/2009	9 (2.0)	0.78	0.8184	2.0164
401	Feather	5/27/2009	11 (2.0)	0.67	0.8180	2.0160
401	Feather	5/27/2009	13 (2.0)	0.54	0.8185	2.0168
401	Feather	5/27/2009	15 (2.0)	0.53	0.8180	2.0182
401	Feather	5/27/2009	17 (2.0)	0.55	0.8179	2.0189
401	Feather	5/27/2009	18+19 (3.5)	0.53	0.8181	2.0187
401	Feather	5/18/2011	1 (1.3)	3.36	0.8631	2.0908
401	Feather	5/18/2011	3 (1.9)	1.76	0.8528	2.0783
401	Feather	5/18/2011	6 (2.1)	1.41	0.8449	2.0668
401	Feather	5/18/2011	9 (2.1)	1.03	0.8401	2.0596
401	Feather	5/18/2011	12 (2.0)	1.00	0.8365	2.0533
401	Feather	5/18/2011	15 (2.0)	15.1	0.8162	2.0147
401	Feather	5/18/2011	18 (1.9)	53.7	0.8161	2.0010
401	Feather	5/18/2011	21 (2.0)	53.6	0.8142	2.0042

^a Feather vane segments ordered distal (oldest)– proximal (newest) (segment length in cm).

^b Blood Pb concentrations in $\mu\text{g}/\text{dL}$, feather and tissue Pb concentrations in $\mu\text{g}/\text{g}$ dry weight.

marked fully grown feathers are identified and a ~2 cm deep margin of trailing vane is cut along the entire rachis and stored in polyethylene bags at room temperature until processing for analysis. At UCSC feather samples are cut into ~2 cm wide sections (perpendicular to rachis) for individual analysis as described by Finkelstein et al. (2010).

3) If a condor is lead poisoned (blood lead > 35 $\mu\text{g}/\text{dL}$), the condor is typically transferred to the Gottlieb Animal Health and Conservation Center (LA Zoo, California, USA) for chelation treatment and observation, and growing primary feathers are marked and measured for potential collection and analysis, as described above. As the principal cause of lead exposure in



Fig. A1. Radiograph of condor 401 taken on 21 June 2010 shows three radio-opaque objects embedded in the wing (birdshot) and one larger radio-opaque object in the bird's digestive tract, later identified as lead buckshot after regurgitation and analysis. Inset panel, lower right: comparison of surgically removed birdshot (left) with the regurgitated buckshot pellet (right).

condors is ingestion of a lead item, whole body radiographs are performed to detect the presence of radio-opaque objects within the bird's digestive tract and assess the need for surgical or other intervention. Radio-opaque objects may be surgically removed or recovered following induced regurgitation.

- 4) When a condor dies of suspected lead poisoning, tissue samples (e.g. bone, kidney and liver) and radio-opaque objects still in the digestive tract are collected from the carcass by veterinary pathology staff affiliated with the California Condor Recovery Program and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Whole growing primary feathers are removed from the carcass and stored in polyethylene bags for potential analysis.

Sample collection details for California condors 286, 375, 401

Condor 286: On 4 March 2009 condor 286 was captured by Ventana Wildlife Society (VWS) field biologists for routine lead exposure monitoring. A blood sample identified the bird as lead poisoned (blood lead value of "High", LeadCare, Magellan Diagnostics) and he was transported to the LA Zoo for chelation treatment. Radiographs revealed 10 birdshot pellets embedded in the bird's tissue. The bird died at the LA Zoo on 11 May 2009 from suspected lead toxicosis, which was later confirmed by veterinary pathologists (Rideout et al., 2012). Five birdshot pellets, samples of liver, kidney, tibiotarsus as well as a growing primary feather (total vane length=32.5 cm) were collected post-mortem by veterinary pathology staff at the Wildlife Disease Laboratories at the Institute for Conservation Research San Diego Zoo Global.

Condor 375: On 26 March 2009 condor 375 was captured by VWS field biologists for routine lead exposure monitoring. A blood sample identified the bird as lead poisoned (blood lead value of "High", LeadCare, Magellan Diagnostics) and she was transported to the LA Zoo for chelation treatment. Radiographs revealed three birdshot pellets embedded in the bird's tissue. One pellet was surgically removed. The condor recovered and a growing primary feather vane sample was collected (total vane length=21.4 cm) on 1 May 2009 prior to release back into the wild.

Condor 401: On 12 April 2009 condor 401 was captured by Pinnacles National Park (PNP, formally Pinnacles National Monument) biologists for routine lead exposure monitoring. A blood sample identified the bird as lead exposed ($> 10\text{ }\mu\text{g/dL}$; Cade, 2007; field value $11\text{ }\mu\text{g/dL}$, LeadCare, Magellan Diagnostics), but below the $35\text{ }\mu\text{g/dL}$ blood lead level threshold for chelation treatment. A growing primary feather was identified and marked for later collection.

On 27 May 2009 condor 401 was recaptured by PNP biologists for routine lead exposure monitoring. A blood sample identified the bird as lead exposed (field value $13\text{ }\mu\text{g/dL}$, LeadCare, Magellan Diagnostics) and the trailing vane of the previously marked primary feather was collected (total vane length=39.3 cm).

On 30 October 2009 condor 401 was recaptured by VWS biologists for routine lead exposure monitoring. A blood sample identified the bird as lead poisoned (blood lead value of "High", LeadCare, Magellan Diagnostics) and he was transported to the LA Zoo for chelation treatment. Radiographs revealed four birdshot pellets embedded in the bird's tissue. One pellet was surgically removed, and the bird recovered and was released back into the wild on 19 November 2009.

On 21 June 2010 condor 401 was recaptured by VWS biologists for routine lead exposure monitoring. A blood sample identified the bird as lead poisoned (blood lead value of "High", LeadCare, Magellan Diagnostics) and he was transported to the LA Zoo for treatment. Radiographs revealed the three remaining embedded birdshot pellets as well as a large object within the bird's gastrointestinal tract, which was regurgitated by the bird within the clinic and collected. A second birdshot pellet was also surgically removed on 22 June 2010. The bird recovered and prior to release back into the wild on 6 October 2010 a growing primary feather was marked by biologists via notching the vane. A vane sample from this marked feather was subsequently collected on 18 May 2011 after the feather was fully grown (total vane length=39.9 cm).

Appendix B

Condor feather growth timeline

As we have noted previously (Finkelstein et al., 2010), estimation of a feather growth timeline depends on accurate feather length measurements, though obtaining accurate calamus and total feather length measurements from restrained live birds can be challenging. Further, the time lag between when a growing vane segment becomes isolated from the blood supply in the proximal calamus region to when it emerges from the calamus sheath and becomes available for sampling is unknown. For condors 375 and 401 (27 May 2009 collection), whose feathers were still growing when collected, we estimated this time lag to be ~ 6 days; i.e. the newly grown proximal feather vane segment might reflect lead in the bird's blood from ~ 6 days before this vane segment emerged from the calamus sheath and was collected. Our time lag adjustment is supported by the observation that the lead isotopic composition from the blood samples

collected during feather growth is not measurably different than the corresponding feather sections for these dates (Fig. 2B).

For condor 286, a growing primary feather was measured when he was hospitalized for clinical treatment of severe lead poisoning on 5 March 2009, which allowed assessment of the feather growth rate until death on 11 May 2009. The feather grew 0.9 cm over this 67 day period, for a growth rate of 0.013 cm/day. This growth rate was dramatically slower than the average rate of 0.441 cm/day determined previously for adult condors (Finkelstein et al., 2010), and is attributed to the moribund condition of condor 286 from lead toxicosis. Therefore, we adjusted condor 286's feather growth rate for the most recently grown 0.9 cm section of feather to 0.013 cm/day while setting a growth rate of 0.441 for the remainder of the feather (i.e. the remaining = 31.6 cm length). Noteworthy is that the lead isotopic composition from the blood sample collected on 4 March 2009 was measurably indistinguishable from the corresponding feather section for this date (Fig. 2B), supporting our adjustments to condor 286's feather growth rate.

The feather from condor 401 (5 May 2011 collection) was fully grown when sampled so adjusting for the time lag between when a growing vane segment was isolated from the blood supply and emerged from the calamus sheath was not necessary. However, the tip of 401's feather had broken off before sampling and the length of the missing section was not known precisely, impacting our ability to determine an accurate total feather length. To address this, we estimated that the missing tip section was 2.6 cm in length, based on prior field measurements of the feather when the tip was still intact (see Fig. 3B).

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