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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 μg/dL), illustrating that ingested shot possess a substantially greater lead poisoning risk compared to embedded shot retained in tissue (blood lead ~20 μg/dL). To our knowledge, this is the first study to use lead isotopes as a tool to retrospectively link wildlife shooting events.

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% HNO_3 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 HNO_3 in closed Teflon vials, evaporated to dryness, and reconstituted in 1% HNO_3 for analysis. Birdshot/buckshot pellets were individually cleaned and then leached in 1 mL 1% HNO_3 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 ^{206}Pb, ^{207}Pb, and ^{208}Pb as previously described (Finkelstein et al., 2003; Gwiazda et al., 1998). Added ^{205}Tl 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 ($^{207}Pb/^{206}Pb$) 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