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Date: June 15, 2008

From: Donald Smith, Ph.D.
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Re: Final report for funded research entitled "Stable Isotopic Fingerprinting of Lead Sources to California Condors in Pinnacles National Monument"

We proposed three objectives in this study: (1) *Perform blood lead monitoring of condors released in the Pinnacles National Monument using stable lead isotopic fingerprinting methods;* (2) *Perform serial feather lead isotopic analyses of known growing feathers from recaptured birds to reconstruct the lead exposure history over the feather growth period (i.e., previous 3-4 months), and;* (3) *Perform lead isotopic analyses of recoverable prey items that condors are observed feeding on, and analyses of small and large caliber and shotgun ammunition for isotopic signature.* In summary, we met or exceeded expectations for achieving these objectives; research supported in full or in part by WNPA resulted in the analyses of blood samples from five pre-release condors from PNM, 16 free-flying condors from PNM and the Ventana Wildlife Society release site (birds from both PNM and VWS co-mingle), and seven free-flying condors from the Hopper Mountain release site (managed by USFWS). In addition, we analyzed feather samples from five free-flying condors from PNM and VWS, and two bullets recovered from a pig carcass that PNM condors were observed feeding on.

These analyses produced valuable new data that greatly extend our understanding of acute lead poisoning events in released condors and further validated the substantial utility serial feather lead isotopic analyses provide to reconstruct the lead exposure history of released condors. Moreover, data from several case studies (condors #306 and 318 presented below) were presented to the California Fish and Game Commission at a special meeting in August, 2007 to consider a partial ban of lead ammunition in condor range in California. These studies presented a convincing

and compelling case demonstrating that lead ammunition was responsible for the lead poisonings in those birds, and as such were instrumental in providing clear scientific evidence supporting lead ammunition as the principle source of lead poisoning in condors. Finally, a sub-set of the data were presented in an oral platform session at a conference on 'Ingestion of Spent Ammunition: Implications for Wildlife and Humans' convened by The Peregrine Fund (Boise, Idaho, May 12 – 15), and they will serve as the basis for a peer-reviewed publication that will be prepared and submitted latter this summer (2008). These results are presented in detail below.

In collaboration with National Park Service (NPS) biologists and staff, we have to date received and analyzed a total of 30 blood samples representing a total of 21 different condors, including five condors prior to their release at Pinnacles National Monument, shown in **Figure 1**. Because the majority, but not all, of analyzed blood samples from free flying condors in California are consistent with the isotopic composition of the lead ammunition analyzed by Church et al. (2006), these data largely corroborate our prior conclusions that lead ammunition is the principle source of lead exposure to released condors in California. Included in these new analyses is a revised two-endmember mixing model that uses the isotopic composition of pre-release PNM condors and ammunition from Church et al. as the two end-members (dashed line in **Figure 1**), which corroborates our previous published model (solid line, **Figure 1**). Notably, there are several blood samples from free-flying PNM condors that do not fit this simple lead-exposure model, suggesting the sources of lead exposure to those birds is either ammunition with an isotopic composition different from what we have measured so far, or from an unknown non-ammunition lead source(s) with isotopic compositions different from our measured ammunition.

Perhaps most important results are those showing that the serial sampling of growing feathers from free-flying condors can be used to reconstruct the lead exposure history over the months preceding feather sampling. As background, we believe that analyses of growing feathers represent an important tool to reconstruct the exposure history for individual condors. Primary flight feathers grow ~ 4.5 mm/d and take 105-120 d (3.5 – 4 months) to fully grow. Thus, analysis of a single feather could represent up to ~30% or more of the birds exposure history within a given year. We analyzed serial feather samples from five different condors totaling more than 70 individual samples (#306, 318, 307, 336, and two feathers from 351). Of particular note are the results from two in-depth case studies on condors #306 and 318, who were lead-poisoned from feeding on a pig carcass shot on a ranch near PNM. These cases studies are presented below. Notably, they were also presented to the California Fish and Game Commission in August 2007, where they played an important role in the Commission's favorable ruling to partially ban lead ammunition in parts of the State. They represent near-ideal cases demonstrating exposures from ingested ammunition and the significant utility of feather analyses to reconstruct that exposure history. Data from the other three birds follows.

Condors #306 and 318: Timeline of exposures and sample collection: On 24 or 25 June 2007, a pig was shot by a rancher on private property several miles east of

PNM. On 25 June 2007, six California Condors, including condor 318, were positively observed feeding on the pig carcass. The carcass was recovered and stored by NPS personnel. On 26 June 2007, condors 306 and 318 (among others) were trapped into the flight pen at PNM. On 28 June 2007, the recovered pig carcass was x-rayed by NPS personnel, and two bullet slugs within the carcass were tentatively identified. On 2 July 2007, whole blood samples were collected from condors 306 and 318. Based on elevated blood lead levels, chelation treatment was initiated at PNM. On 3 July 2007, condors 306 and 318 were transported to the LA Zoo for a full chelation regimen, and then transported back to PNM following treatment. On 29 July 2007, a second set of blood samples were collected from 306 and 318. Serial feather samples were collected from a growing flight feather from each bird. The bullets were recovered from the pig carcass (see **Figure 2**), and the blood, feather, and bullet samples were transported to UC Santa Cruz for analyses.

Condors #306 and 318 case studies: Lead isotopic signature of bullets recovered from the pig carcass. Lead bullets recovered from the pig matched the upper isotopic range of ammunition measured by Church et al. (*Environ. Sci. Technol.* **2006**, 40, 6143). These bullets appear to have originated from .22 caliber ammunition, which was not sampled by Church et al. (2006). This provides important additional evidence that the isotopic signatures of ammunition reported by Church et al. reflect ammunition used in central California. To demonstrate that the ingestion of lead bullets (or fragments of bullets) by California Condors is sufficient to cause the very elevated blood lead levels observed in many free-flying condors, we subjected the two lead bullets recovered from the pig carcass to a chemical leaching process designed to loosely approximate the condor's upper GI tract conditions. In general, the amount of lead leached from bullets or bullet fragments within the GI tract would be expected to vary with the number of fragments, their surface area and retention time, and the chemical and physical properties within the GI tract. Here, we leached bullets in 0.5N HCl at 40°C for durations of 3, 18, 24, or 120 hours. Results show that after 3 hours a total of 14,500 µg Pb were leached from the bullets, increasing to 25,200 µg Pb leached after 24 hours (**Figure 2**). If one assumes an average condor blood volume of 552 mL (based on an average body weight of 8.5 kg and an average blood volume of 6.5% of body weight), and a GI lead absorption efficiency of 10% (i.e., 10% of soluble lead in the GI tract is absorbed), the amount of lead leached after only 3 hours would be sufficient to produce a blood lead concentration of ~263 µg/dL or 2,630 ng/mL (i.e., 14,500 µg Pb/552 mL blood = 26.3 µg/mL = 2630 µg/dL * 0.1 absorbed), assuming all of the absorbed lead entered the blood stream. This amount of lead is clearly sufficient to cause extreme lead poisoning.

Feather and blood analyses for condors 306 and 318: A summary of blood lead concentration and isotopic composition data for condors 306 and 318, including data from Church et al. (2006) on the blood lead values for these birds prior to their release into the wild provides clear evidence that the birds acquired a blood lead isotopic composition following exposure to the contaminated pig carcass (PbIC of recovered ammunition plotted on the right y-axis) (**Figure 3**). After chelation treatment, the blood lead values declined in concentration and began showing an isotopic composition that was moving back towards the isotopic composition of background lead in California.

Serial feather samples from condor 306 provide clear evidence of this acute lead exposure event that occurred ~ 35 days prior to feather sampling (re-constructed timeline based on feather growth rate of ~4.5 mm/d (Snyder et al., *The Condor* **1987**, 89(3), 468) (**Figure 4**). The feather lead concentration starts (distal tip of feather) at very low lead levels of <0.5µg/g, and sharply increases to >3µg/g with exposure. The feather isotopic signature reflects background lead in sections with very low lead concentrations and then sharply changes to a lead isotope signature that asymptotes at the isotopic signature of the peak blood level value and the bullets recovered from the pig.

Serial feather samples from condor #318 also provide clear evidence of an acute lead exposure event that occurred ~ 35 days prior to feather sampling, coinciding with feather sections 12 – 16 (**Figure 5**); re-constructed timeline based on feather growth rate of ~4.5 mm/d (Snyder et al., *The Condor* **1987**, 89(3), 468). These feather samples also provide evidence that condor 318 had suffered a prior lead exposure event some time before the growth of this feather. The feather lead concentrations declined slightly from the first (oldest) feather section to low values of <0.5µg/g, and then increased dramatically in concentration in newer feather sections 12 – 14 (**Figure 5**). There was a small coinciding change in the feather isotopic signature, which changed to values matching the isotope signature of the blood and recovered bullets in the newest feather sections.

Feather and blood analyses from 336, 307, and 351: For condor #336, blood lead levels at time of feather sampling indicate a modest exposure of 16 µg/dL. The blood lead levels preceding this (measured by PNM staff) were collected in July, 4.5 months before, and at ~30 – 45 µg/dL indicate a moderate exposure. Interestingly, the feather at the oldest distal section shows clear evidence of a high exposure (**Figure 6**). The feather lead level of 7.3 µg/g in the most distal part is much higher than the feather lead for birds #306 and 318 (above), who had blood lead levels of 80 – 160 µg/dL at their probable peak exposure. Also, the most distal part of the feather likely grew about 3 – 3.5 months before sampling (i.e., mid-December), putting the exposure at about 4 months prior to feather sampling, or mid-August, which is after the blood lead values measure in June/July. Thus, this case provides evidence of a very elevated exposure that was not captured by the blood lead levels, and thus would have otherwise gone undetected. The lead isotopic composition of this presumed exposure 4 months before feather sampling matches the isotopic composition of ammunition from Church et al. (2006), and interestingly there is a rapid decline in feather lead levels but a much slower 'recovery' in the isotopic composition of the feather – this could suggest that the bird is accumulating lead with an isotopic composition that may 'look' more like ammunition.

Feather data for condor #307 can also be used to infer its past exposure history (**Figure 7**). In fact, working backwards in time, the blood lead level at the time of feather sampling was 14 µg/dL, but 2 – 3 weeks prior to that it was 40 – 65 µg/dL. The feather lead shows a 'bump' of an increase at section 5, and again at section 7 – 8 that is matched by changes in the feather isotopic composition trending lower towards the

isotopic composition of ammunition from Church et al. (2006) (**Figure 7**). Importantly, the most recent blood lead value (14 µg/dL) does not match the feather lead level, which continues to go up at the most recent feather section sample. Thus, these data may suggest a lag of about 2 – 3 weeks between a blood lead value and when the feather segment that was growing at the time of the blood lead becomes available to sample.

For condor 351, two feathers were analyzed, one collected December 2006 and the other November 2007. The 2006 sample provides evidence of a very modest exposure around section 10 – 11 (**Figure 8a**). The 2007 sample is even more interesting because the lead level is low and unchanging across the entire feather, but the lead isotopic composition seems to change in some cycling pattern within the range of background lead isotopic composition (**Figure 8b**). On the surface, and assuming the blood isotopic composition at the second collection was at one point in the past like the lead isotopic composition of the feather at the first collection, it shows that the blood isotopic composition will recover back to the background isotopic composition, suggesting in this case that significant lead stores may not be accumulating in bone and then serving as important internal sources of lead and lead isotopic compositions as may occur in humans. The cycling pattern in the isotopic composition in this feather may reflect the birds feeding episodes. This is a case where, if available, GPS based and visual observational data for this bird over the period when the feather was growing may be useful in understanding the possible reasons for the cycling in feather isotopic compositions.

In conclusion, these data more than doubled the existing data on lead isotopic compositions in condors released in California. They generally corroborate prior suggestions that incidental ingestion of lead ammunition is the principle source of lead exposure in released condors, and they have made substantial contributions to recent deliberations by the California Fish and Game Commission to ban lead ammunition for hunting in some parts of the State. Moreover, these data strongly support the value of routinely sampling growing feathers to monitor the lead exposure history of free-flying California condors. These feather lead and isotopic composition data suggest there is a temporal lag of ~ 2 weeks between the blood lead level and isotopic composition and then this blood lead appears in a growing feather (vane) that is available for field sampling. Additional research is warranted, however, to better link specific cases of exposure with recovered ammunition, as was done here with the cases studies on condors #306 and 318. In addition, in order to realize the full potential that feather sampling could provide to reconstruct exposure history, further research is necessary to determine the relationship between blood lead concentrations and feather lead concentrations, since better understanding of this relationship would facilitate use of feather lead concentrations as a biomarker for predicting risk of toxic effects.

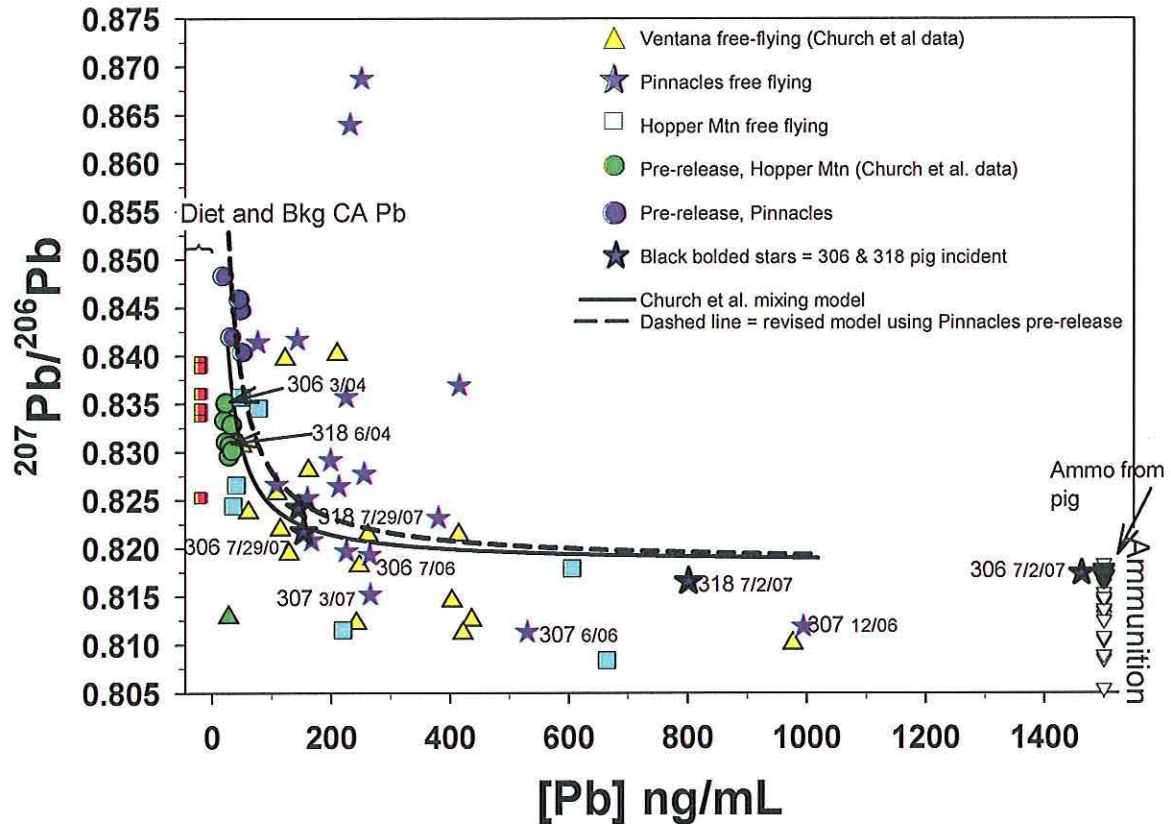


Figure 1. Blood lead concentration versus blood lead isotopic composition (PbIC) ($^{207}\text{Pb}/^{206}\text{Pb}$ ratio) in condors in California (legend inset identifies symbols). Also shown are the PbIC of ammunition from Church et al. (2006; inverted grey triangles on right y-axis), and the PbIC of ammunition (inverted black triangles, right y-axis) recovered from the pig carcass relevant to the cases studies for condors #307 and 318. Lines reflect two mixing models; the solid line is the model from Church et al. (2006), while the dashed line is a revised model using the data from pre-release condors from PNM (solid blue circles) as the starting background values before release into the wild.

Figure 2. Amount of lead leached from the bullets recovered from the pig carcass relevant to condor #306 and #318 case studies (see text). Best fit line based on the equation $f=a*x/(1+b*x)$. Inset shows pictures of the two bullets recovered from the pig carcass near Pinnacles National Monument. Bullet dimensions suggest this is .22 caliber ammunition.

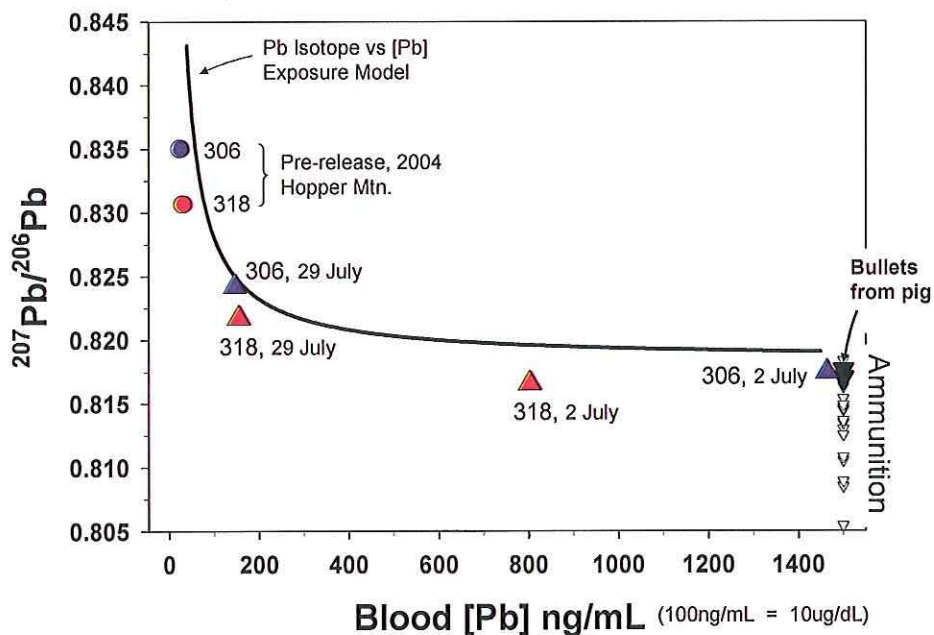
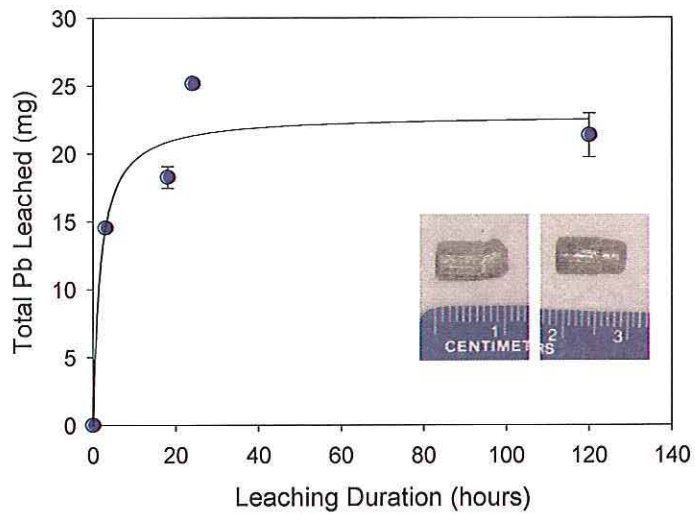
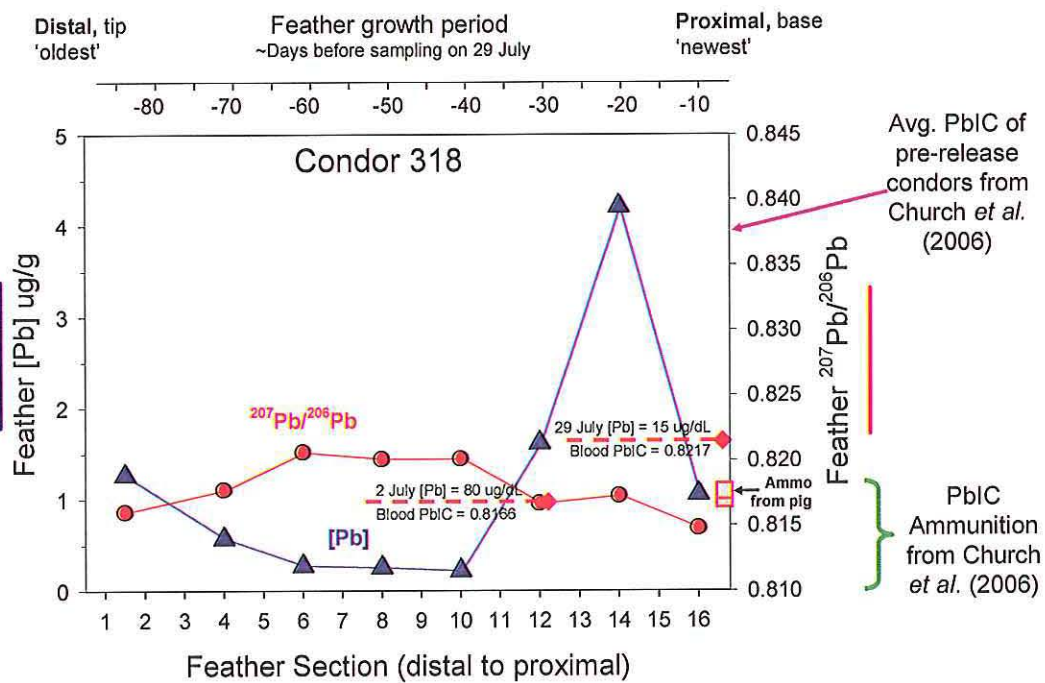
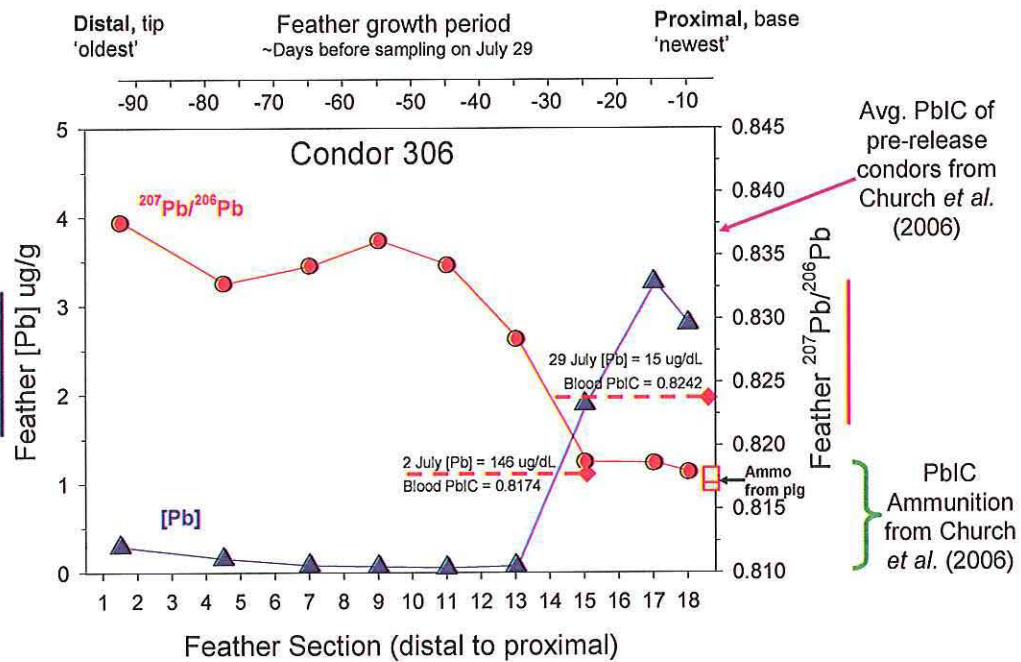


Figure 3. Lead concentration (x-axis) versus isotope signature ($^{207}\text{Pb}/^{206}\text{Pb}$ ratio, y-axis) in blood samples from condor #306 (blue symbols) and condor #318 (red symbols); dates of sample collection indicated next to symbol – circles show pre-release values at Hopper Mountain reported in Church et al. (2006). Also shown are the isotope signatures of lead bullets recovered from the pig carcass July 2007 (solid black inverted triangles) and their comparison with the isotope signature in lead ammunition collected in central California (small inverted gray triangles, Church et al., 2006). The solid line shows the lead exposure model, which is based on exposures to lead from 'background' lead measured in pre-release condors and an elevated exposure from ammunition (isotope signature matching the upper range of measured ammunition).



Figures 4 and 5. Feather lead concentration (left y-axis) and isotopic composition (right y-axis) of serially sampled growing flight feathers from condors #306 and #318. Also shown are the PbIC of ammunition recovered from the pig carcass (ammo shown in Figure 2 above), the PbIC of blood samples collected on 2 July and 29 July, 2007, and the PbIC of the average pre-release condor blood values and of the range of ammunition from Church et al. (2006).

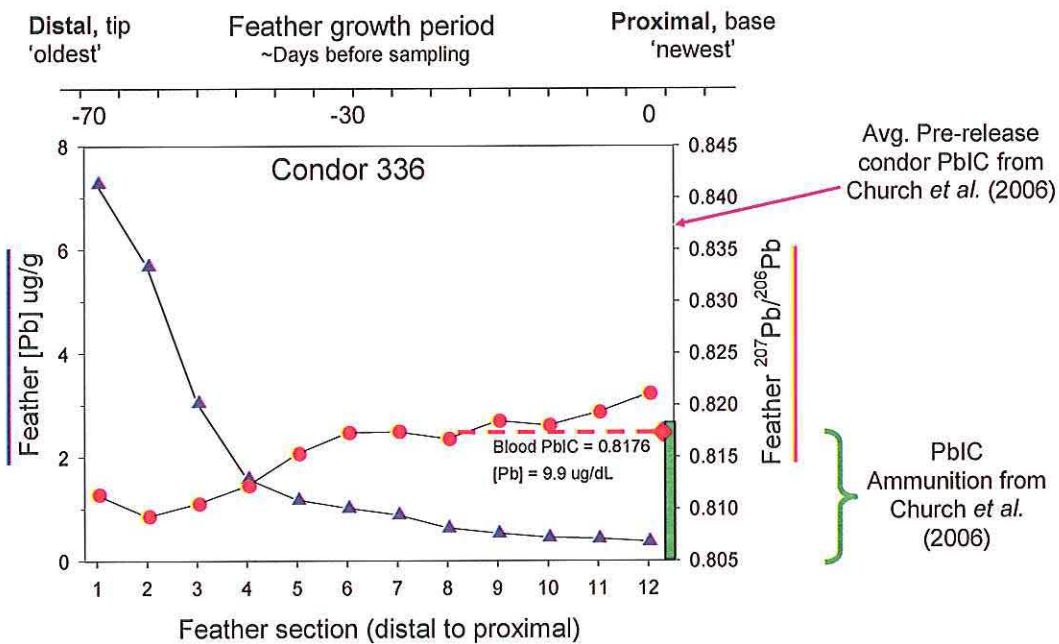
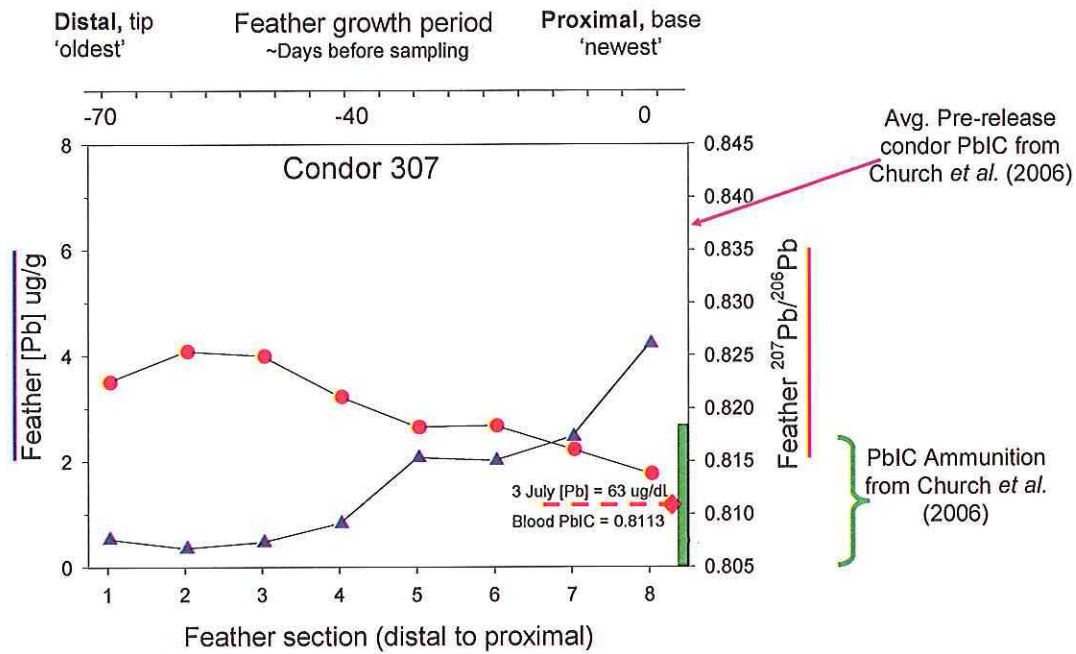


Figure 6 and 7. Feather lead concentration (left y-axis) and isotopic composition (right y-axis) of serially sampled growing flight feathers from condors #307 and #336. Also shown are the PbIC of blood samples collected on the date that the feather samples were collected, and the PbIC of the average pre-release condor blood values and of the range of ammunition from Church *et al.* (2006).

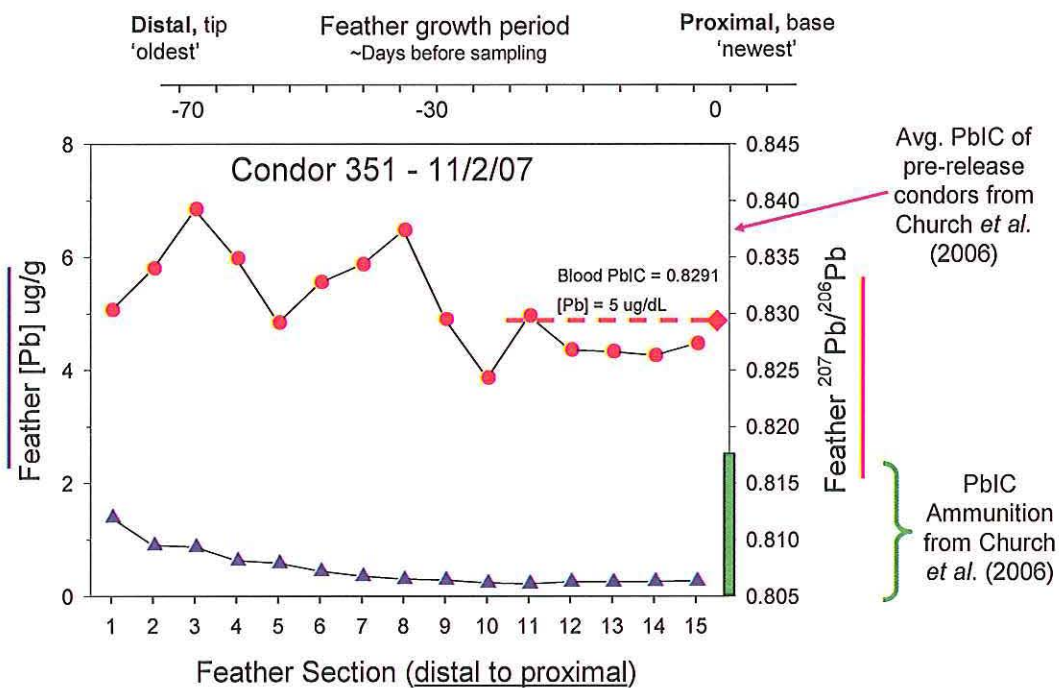
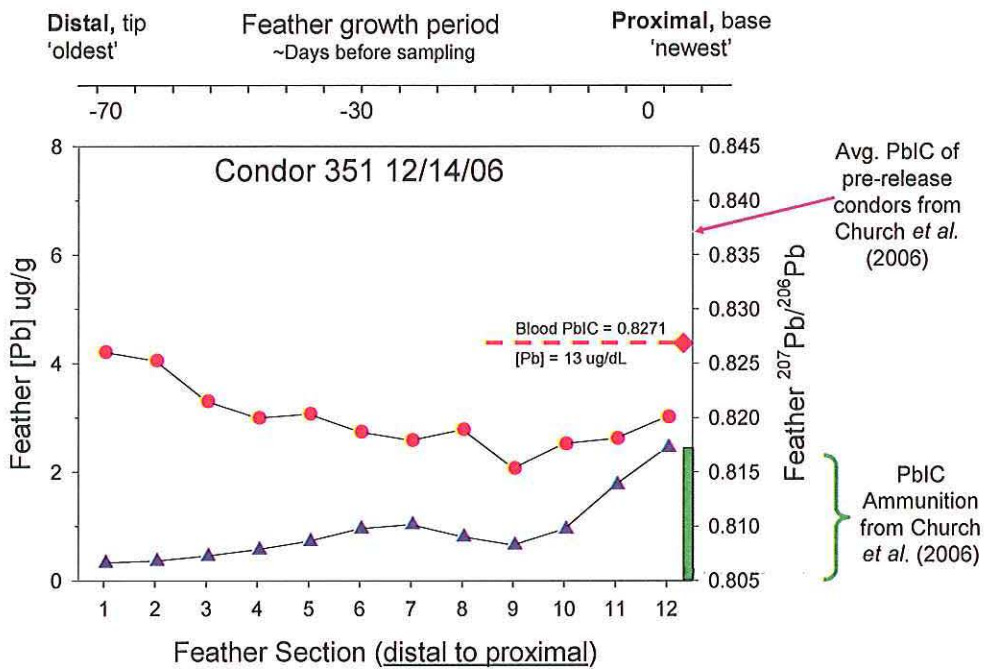


Figure 8a, b. Feather lead concentration (left y-axis) and isotopic composition (right y-axis) of serially sampled growing flight feathers from condor #351 (samples were collected from two different flight feathers at two different times). Also shown are the PbIC of blood samples collected on the date that the feather samples were collected, and the PbIC of the average pre-release condor blood values and of the range of ammunition from Church *et al.* (2006).