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LEAD CONCENTRATIONS WITHIN THE CONDOR SKELETON: ADVANCING BIOMARKERS OF LEAD EXPOSURE HISTORY AND POISONING

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Abstract

Lead concentrations within the condor skeleton: advancing biomarkers of lead exposure history and poisoning

by

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Lead (Pb) poisoning is a global problem among avian species, including the endangered California Condor (*Gymnogyps californianus*). Condors are highly monitored and frequently lead poisoned and may serve as a model species to investigate Pb exposure biomarkers. Bone Pb levels are a well-recognized biomarker of Pb exposure and related health effects in humans but have not been used widely in avian species. My overarching objective is to further establish biomarkers of lead exposure history and poisoning in avian species using bone Pb concentrations. In chapter 1, I investigated whether bone Pb differed between and within condor bones, depending on Pb exposure history between epiphyses, mostly composed of trabecular bone type, and diaphysis, mostly composed of compact bone type. In chapter 2, I determined if Pb levels in different bones can be used as biomarkers of recent and cumulative Pb exposure history and examined the effects of Pb exposure on bone mineral density and potential risk for bone fracture. In chapter 3, I used Pb stable isotopic composition to inform about Pb uptake into multiple bones and bone regions. My results showed that Pb concentrations were ~3-fold higher in epiphysis than diaphysis of tibiotarsus in

condors that died of Pb toxicosis but less than 2-fold in condors that died of other causes. A discriminant analysis using bone Pb concentrations correctly classified 17 out of 18 birds as to whether they were Pb poisoned at the time of death, suggesting that bone Pb, particularly tibiotarsus epiphysis proximal, can be used as an additional piece of information to inform recent Pb exposure. Bone Pb levels, particularly in tibiotarsus diaphysis, were associated with time in the wild, consistent with prior studies in humans showing that bone Pb levels in long bones reflect long term cumulative Pb exposure. I also found a modest negative association between bone Pb and bone mineral contents in epiphyses of long bones, suggesting that bone Pb may be associated with a reduction in bone mineral. Finally, using stable lead isotopes, I found that there was ~10-fold difference in the rate of Pb incorporation between the tibiotarsus proximal epiphysis and diaphysis following a Pb exposure event. In conclusion, bone Pb levels in condors, and by extension other large avian species, appear to be a valuable biomarker of both recent acute and cumulative Pb exposure, and may help inform Pb poisoning status at the time of death.

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Chapter 1) Introduction

Lead (Pb) poisoning is a global problem in wildlife and in particular in avian species. It is estimated that millions of birds die annually from Pb poisoning (de Francisco, Ruiz Troya and Agüera, 2003). In California, lead toxicosis was the cause of death for 17% of wild eagles and turkey vultures (Kelly *et al.*, 2014). Furthermore, in California and Arizona, lead toxicosis was the cause of death for 28% of adult California Condors (*Gymnogyps californianus*) (Rideout *et al.*, 2012). Given its endangered status, California Condors are highly monitored, with radiotransmiters, routine monitoring for lead poisoning, diseases, and health (USFWS, 2014), which makes them a good model species to study Pb poisoning in large wild birds.

The main exposure source in wild condors is spent ammunition (Church *et al.*, 2006; Finkelstein *et al.*, 2012). Lead is ingested by birds while feeding on carcasses that were shot with Pb ammunition (de Francisco, Ruiz Troya and Agüera, 2003). Feeding on carcasses shot with Pb ammunition is particularly a problem among scavengers, including condors and may have debilitating or lethal consequences for them (Pattee *et al.*, 1981; Janssen *et al.*, 1986). Despite the fact that clean food is supplied by management teams, released birds have not remained strictly dependent on them (Meretsky *et al.*, 2000), condors have been reported to feed on proffered carcass for an average of 10% of the number of days they were in the wild (Bakker *et al.*, 2016). After Pb-contaminated meat is consumed by condors, the particles of Pb enter the digestive tract and begin to dissolve and Pb is absorbed into the bloodstream. Once in

circulation, Pb is distributed to the rest of the body and can possibly cause physiological or behavioral effects (de Francisco, Ruiz Troya and Agüera, 2003).

Several authors have studied the effects of Pb toxicity in birds. For instance, willow grouses presented disturbed eating behavior after being treated with Pb pellets through ingestion (Fimreite, 1984). Circulating white blood cells (WBC) and Spleen Plaque-forming cell counts declined significantly in male mallards exposed to Pb by either natural ingestion or intubation (blood Pb concentration of 4 μg/dL) (Rocke and Samuel, 1991). There have also been reports of Pb intoxication in different species, such as Eurasian Gryphon (Mateo, Taggart and Meharg, 2003), Spanish Imperial Eagle (Mateo *et al.*, 2001; Pain *et al.*, 2005), Red Kite (Mateo *et al.*, 2001), Laysan Albatross (Finkelstein, Gwiazda and Smith, 2003), Griffon Vultures (Mateo *et al.*, 1997), mallards (Rocke and Samuel, 1991) and others (Fisher, Pain and Thomas, 2006).

Treatments for Pb intoxication include removing fragments by endoscopy (Walters *et al.*, 2010), and chelation in cases where condors present blood Pb levels >35 ug/dL (USFWS, 2014). Chelation reduces circulatory Pb levels (Smith, Bayer and Strupp, 1998; Walters *et al.*, 2010), although it is unclear how chelation affects bone Pb, in particular in avian species.

1.1 Current monitoring of Pb exposure in California condors and potential for avian bones as biomarkers of Pb exposure

Due to its endangered status, condors are more heavily monitored than other large birds. Board-certified pathologists perform a necropsy in condor carcasses recovered (Rideout *et al.*, 2012) and condors are routinely monitored for Pb exposure.

Currently, the gold standard to diagnose whether a condor died of Pb toxicosis is blood and liver Pb concentrations (Rideout *et al.*, 2012). While it is known that Pb poisoning is the primary cause of death (CoD) for 28% of adult condor mortalities (Rideout *et al.*, 2012), this number is likely underestimated. Liver Pb concentration is measured during routine necropsy and can only be used if the carcass was recovered fresh in the wild and not scavenged (Rideout *et al.*, 2012). Carcasses found heavily scavenged often have most of the soft tissue missing, so only bone and feathers are recovered.

In practice, however, Pb exposure morbidity and mortality risk is likely better associated with a condor's comprehensive Pb exposure history (both cumulative and acute), as it has been shown in humans (Hu *et al.*, 1994, 1996; Cheng *et al.*, 2001). However, blood Pb levels may be of limited use to assess long term exposure in California Condors since Pb has a relatively low half-life in blood (~13 days) following an exposure event (Fry and Maurer, 2003). Therefore, the current standard of biannual blood Pb monitoring may capture only ∼10% of a condor's annual exposure history. On the other hand, the analysis of Pb in sequential feather segments has proven useful in evaluating a condor's Pb exposure history over a period of several months (Church *et al.*, 2006; Finkelstein *et al.*, 2010, 2012). Segmented feather analysis allows for the estimation of timeframes and severity of exposure, enabling an accurate estimation of the historic of exposure (Finkelstein *et al.*, 2010, 2012).

Bone Pb concentration has been recognized as a biomarker for Pb toxicity in humans and has been shown to be a more accurate predictor of Pb-related health effects than the other aforementioned markers (Silbergeld, Schwartz and Mahaffey, 1988; Hu,

1998; Hu, Rabinowitz and Smith, 1998; Campbell and Auinger, 2007; Beier *et al.*, 2013). Different studies have investigated the kinetics of Pb in the bones, and investigated the remobilization of bone Pb back to the blood (Wittmers *et al.*, 1988; Rabinowitz, 1991; Aufderheide and Wittmers, 1992; Smith, Osterloh and Flegal, 1996; Fleming *et al.*, 1999). Since bone Pb has a longer half-life (years) than soft tissue (several weeks) and blood (days to weeks) Pb, bone Pb concentration can reflect long-term exposure, whereas soft-tissue and blood Pb concentration indicates more recent exposure. There is evidence that bone Pb is a more useful indicator than feather Pb for cumulative Pb exposure, since it reflects long term exposure (García-Fernández *et al.*, 1997; Pain *et al.*, 2005; Finkelstein *et al.*, 2010, 2014). Also, in different species of birds, bone Pb concentration ($< 15\ \mu g/g$) from the ingestion of Pb spent ammunition was shown to be related to a reduction in bone density (Gangoso *et al.*, 2009; Álvarez-Lloret *et al.*, 2014).

1.2 Avian bone structure and bone mineral turnover

Birds have developed extremely lightweight skeletal systems that help aid in the generation of lift and thrust forces as well as helping them maintain flight over a long period of time (Kian, 2013). Two general features were acquired by the skeleton of birds during the evolution of flight: rigidity and lightness. The rigidity is the result of various fusions of neighboring bones, e.g., fused finger bones, wrist bones, and pelvic girdle bones (Podulka, Rohrbaugh and Bonney, 2001). Lightness comes from cavities or spaces within some specialized pneumatized bones as a bird grows, these spaces contain air sacs, which connect to the respiratory system (Podulka, Rohrbaugh

and Bonney, 2001). Air spaces in skull bones are connected to nasal passageways, whereas other bones are connected to either the air sac or the lungs directly (Podulka, Rohrbaugh and Bonney, 2001).

Most bones have basic architecture composed of an outer cortex and inner medullary cavity. Cortical areas are comprised of compact bone that forms a rigid outer shell that resists deformation. The inner medullary cavity is comprised of a trabecular meshwork of spongy bone and provides strength by acting as a complex system of internal struts (Blanton and Biggs, 1968). In human adults, the mean density of fresh cortical bone has been found to be ~1.85 g/cm^3, and that of trabecular bone has been found to be 1.08 g/cm^3 (Blanton and Biggs, 1968). Long bones of the extremities, such as the femur, are composed of a hollow shaft, or diaphysis, a flared cone-shaped metaphyses below the growth plates, and rounded epiphyses above the growth plates (Clarke, 2008). Bones and regions within a bone differ in the relative amounts of compact and trabecular portions. For instance, in humans, vertebrae are composed of ~75% of trabecular bone, whereas diaphysis of femur is composed of ~95% of cortical bone and femur epiphyses are composed of ~50% of trabecular and 50% of cortical bone (Clarke, 2008).

In order to maintain strength, the mineral portion of bones is constantly being remodeled by replacing micro-damaged bone with newer, healthier bone (Clarke, 2008). The rate of this bone remodeling, or turnover rate, depends on bone type. In humans, the turnover rate of compact bone is 0.23% per month (Clarke, 2008), whereas the rate of trabecular bone turnover is about 2% a month (around 10-fold higher than

compact) (Becker, 2001). In White Leghorn hens (*Gallus gallus domesticus*), bone mineral turnover rates are somewhat higher than in humans, with the turnover of cortical bone of ~0.7% and of trabecular bone of ~4% per month, as determined by radio-isotope measurements for femur and tibiotarsus (Hurwitz, 1965).

As with the naturally occurring mineral turnover in bone, bone Pb turnover rates are different between compact and trabecular bone. The difference in Pb uptake rate is likely attributed to the differing bone surface areas and bone mineral turnover rates of these two bone types (Hu, 1998). The half-life of Pb in trabecular bone Pb pool is much shorter than that of the compact pool (Schutz *et al.*, 1987), in humans Pb half-life was found to be of $t_{1/2} > 5 - 10$ years for cortical and $t_{1/2} > 1$ year for trabecular bone (Rabinowitz, Wetherill and Kopple, 1976; Ranstam, Schütz and Skerfving, 1987; O'Flaherty, 1993). The different bone Pb half-lives in compact and trabecular bone affect Pb accumulation and concentration in these types of bones, since Pb is more rapidly exchanged to and from trabecular bone, the Pb concentration in this type of bone tends to reflect a more recent exposure when compared to compact bone. Over the human lifetime, Pb accumulation is substantially higher in the compact portions of tibia than in other bones, increasing with age (Wittmers *et al.*, 1988; Aufderheide and Wittmers, 1992).

An association between bone Pb concentration and age was found in vultures, which is consistent with Pb accumulation in bone over time. The association with age is due the slow rate at which Pb is released from bones, but shows that multiple exposure effects accumulate during a bird's lifespan (Kendall and Scanlon, 1978;

Gangoso *et al.*, 2009). The accumulation of Pb in bone with age depends on bone type, Pb accumulation is higher in compact than trabecular bone (Wittmers *et al.*, 1988; Aufderheide and Wittmers, 1992).

1.3 Assessment of bone Pb and negative health effects in birds

Existing studies of lead exposure in other avian species use various bones to determine bone Pb concentration. The femur is the most commonly used bone (Álvarez-Lloret et al. 2014; Svanberg et al. 2006; Pain et al. 2005; Mateo et al. 2003; Eeva et al. 2000; Wayland et al. 1999), but other studies used tibiotarsus (Hontelez et al. 1992; Garcia-Fernandez et al. 1997; Janiga & Žemberyová 1998) and humerus (Merchant, Shukla and Akers, 1991; Svanberg *et al.*, 2006; Mateo *et al.*, 2007; Gangoso *et al.*, 2009). The lack of consistency in bone choice does not allow for studies to be directly compared in terms of bone Pb concentrations found.

Mateo et al. (2003) have studied the Pb concentration in different bones, in wild birds of prey exposed to spent ammunition in Spain. The authors found a significant correlation between the Pb levels in the humerus and femur (R = 0.956, Mateo et al. 2014). However, studies with swans and eagles found important differences in bone Pb concentrations across the skeleton of the birds, with tibiotarsus epiphysis proximal having the highest Pb concentration among all bones and also that epiphyses had generally higher Pb concentrations than diaphyses (Ishii et al. 2018).

The skeleton is recognized as an important target organ system for Pb toxicity (Pounds, Long, and Rosen 1991). Among the effects of Pb in bones, the reduction in bone growth has been reported. For instance, in rats, exposure to Pb acetate in the

drinking water was related to the reduced bone growth and strength during the pubertal period (Ronis, 2001).

There is some evidence that Pb is associated with reduction in bone density, e.g., in humans (Klein and Wiren, 1993; Hamilton and O'Flaherty, 1995; Khalil *et al.*, 2014) and in rats (Beier *et al.*, 2013). There are only a few studies that investigate the effects of Pb toxicity in bird bones, although there are several that measure bone Pb concentration in birds. An inverse correlation between bone Pb concentration and bone mineral density was found in Egyptian vultures that ingested spent ammunition (Gangoso *et al.*, 2009), however it was limited by a small number of cases (n = 20), and the inverse relationship seems to be driven by a few data points. The reduction in bone mineral might make bone more susceptible to bone fractures, possibly caused by a reduction in the bone mineralization in the bones (Campbell and Auinger, 2007). The increased number of fractures represents a potential threat to the birds, since they could reduce its motion ability (for fractures in the legs or wings for instance) and could ultimately lead to their death.

1.4 Thesis objectives

The extent to which bone Pb can be used to assess if Pb contributed to a bird's morbidity/mortality is unknown. Thus, there is a need for a more comprehensive Pb exposure biomarker that accurately reflects a condor's recent and chronic exposure history. *The overarching objective of my dissertation is to further establish bone Pb concentrations as biomarkers of Pb exposure history and poisoning in avian species.*

The main objective presented in Chapter 2 is to determine the extent that Pb concentrations vary within the condor skeleton, and how skeletal Pb levels vary within birds with different Pb exposure histories. By first thoroughly evaluating several bones from a single condor, I was able to select nine bone regions that were evaluated in 11 condors. This evaluation was used to guide the selection of bone regions that had the potential of being diagnostic of Pb exposure for further analysis in the remaining of the study.

In Chapter 3, I had three main objectives. First, determine if bone Pb levels in three different bones/bone regions can serve as predictive biomarker of Pb poisoning that can help diagnose whether birds died of Pb toxicosis or not. Second, determine whether bone Pb levels were associated with a decrease in bone mineral content in condors. And third, determine whether cumulative Pb exposure, as reflected in bone Pb levels, was associated with monitoring and life history variables that are measured in free-flying birds.

In Chapter 4, I used Pb stable isotopic composition (PbIC) to inform about Pb toxicokinetics in bone, in particular to estimate the Pb incorporation rate into bones during bone mineral formation following a Pb exposure event. Segmented feather analysis was used to assess a condor's history of exposure in the few months prior to death and analysis of PbIC in feather and bone was used to estimate Pb uptake into bone after exposure. My findings represent one of the first estimations of Pb incorporation rates into condor bones using stable lead isotopes.

I present some concluding remarks and overall observations in Chapter 5. Bone Pb is a promising biomarker that could provide an additional piece of information in determining a condor's Pb exposure history. My findings could be used in other avian species as well, including bald and golden eagles and vultures, where the impact of Pb exposure is not well known.

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Chapter 2) Lead concentrations in epiphyses and diaphyses of condor bones as an indicator of exposure

2.1 Introduction

Lead (Pb) poisoning has been identified as the most important cause of mortality contributing to the decline in California Condor populations in the latter part of the 20th century (Snyder and Snyder, 1989). By 1982, only 22 condors (in the wild and in captivity) existed in the world. As a result, captive breeding programs were started, and by mid-1998, the total number of condors had increased to >150, and by early 1999, 88 birds had been released into the wild (Meretsky *et al.*, 2000). Since then, the captive breeding and release program has increased the total population, as of December 2017 the total population was 463 birds, of which 260 were in the wild (USFWS 2017).

While Pb poisoning is the primary threat to the recovery of California condors (Finkelstein *et al.*, 2012), the full impact of Pb poisoning on condor mortality is likely underestimated. It is not always possible to determine a definitive cause of death (CoD) because carcasses are recovered from the wild without available blood or liver tissue for analysis, and blood and liver Pb concentrations are currently the gold standard to determine whether a condor died of Pb toxicosis (Pain, Fisher and Thomas, 2009). In birds captured alive, diagnosis of Pb poisoning is based on behavioral signs in combination with blood Pb concentration. In birds found dead in the wild, the presence of Pb objects in the gastrointestinal (GI) tract may be used as evidence for diagnosis,

but a positive confirmation can only occur through the measurement of tissue (liver or kidney) Pb concentration (Pain, Fisher and Thomas, 2009). In order for liver Pb to be accurately measured, the carcass needs to be recovered fresh. When condors die in remote locations, this is often hard to accomplish. Furthermore, in cases where condor carcasses are heavily scavenged, most of the soft tissue is missing and only bone and feathers are recovered. Of 82 adult condor carcasses recovered from the wild between 1996 and 2010, 23 died of Pb toxicosis, however, for 20 (24%) it was not possible to diagnose a cause of death due to the carcass being scavenged, too autolyzed for evaluation, or no specific cause or mechanism of death was determined. The relatively high number of condors with no diagnosed cause of death suggests that a larger proportion of condors may have died of Pb toxicosis.

Lead levels in bone have been used successfully as a Pb exposure biomarker in humans and other mammals (Hu, 1998). Lead accumulates in bone over periods of weeks to years, and is known in humans to be a good biomarker of chronic Pb exposure and Pb toxicity (Hu, 1998; Barbosa *et al.*, 2005). It has been found that over the human lifetime, Pb accumulation is substantially higher in the compact portions of tibia than in other bones, increasing with age (Wittmers *et al.*, 1988; Aufderheide and Wittmers, 1992). Those studies have also shown that Pb concentration patterns across bones in humans differ depending on age; for instance, in the age range of 14 – 20 years, skull bone Pb levels were up to 2x higher than levels in rib bone, while humans 21 – 35 years old, the Pb concentrations in these two bones were roughly the same (Wittmers *et al.*, 1988). There are also reports that bone Pb can be used as a predictor of Pb's health

effects. For instance, an association between bone Pb levels and prospective development of hypertension has been found in humans (Hu *et al.*, 1996; Cheng *et al.*, 2001), and child intelligence (IQ) has been shown to be negatively associated with bone Pb levels (Wasserman *et al.*, 2003). In birds, Pb concentrations in bones have been found to be associated with an increased risk of bone fractures (Gangoso *et al.*, 2009). These findings indicate that bone Pb concentrations may provide a useful tool for assessing Pb exposure and possibly for determining whether Pb poisoning contributed to morbidity and mortality in condors (and other avian species).

In non-industrial settings, Pb uptake into the body takes place primarily via the GI tract (Ranstam, Schütz and Skerfving, 1987). Absorbed Pb is distributed to other organs through the circulation and blood plasma. The ability of Pb^{2+} to substitute Ca^{2+} in biomolecules and in bone mineral has been observed in *in vivo* and *in vitro* models (Barton, 1984). Among the soft tissues, the highest Pb concentrations are reached in the liver and kidneys, though the majority of body Pb is contained within the skeleton; in humans the skeleton contains more than 90% of the total burden of Pb in the body (Skerfving and Bergdahl, 2007). Lead uptake into bone depends on blood perfusion in bone and bone mineral formation, since only a very small fraction of Pb can substitute Ca already present in the bone matrix (O'Flaherty, 1993). Lead residency time is usually much longer in bones than in soft tissues, for instance, in humans Pb has a half-life of 40 days in soft tissue and of over 300 days in bones (Rabinowitz, Wetherill and Kopple, 1976). Therefore, kinetically, bone has a much slower turn over Pb than soft tissues (Fleming *et al.*, 1999).

Bone is a connective tissue with mineralized components that is extremely hard in order to provide mechanical support for the body, protection for the soft structures of the body, attachment of muscles that move the skeleton, and to serve as a storehouse for Ca and phosphorus (P) (Ross, Kaye and Pawlina, 2003). The skeleton is divided architecturally into two morphologically distinct bone types – compact and trabecular bone. Compact bone comprises the cortex of long bones and has a relatively dense bone matrix (0.9 g/cm^3 in White Leghorn chickens), whereas trabecular bone has a mesh structure and a relatively less dense composition (0.2 g/cm^3 in White Leghorns) (Jendral *et al.*, 2008). Long bones of the extremities, such as the femur, are composed of a hollow shaft, or diaphysis, flared cone-shaped metaphyses below the growth plates at both ends of the bone, and rounded epiphyses above the growth plates (Clarke, 2008). Notably, bones and regions within a bone differ in the relative amounts of compact and trabecular portions. For instance, in humans, vertebrae are composed of ~75% of trabecular bone, whereas the diaphysis of the femur is composed of ~95% of compact bone and femur epiphyses are composed of ~50% of trabecular and 50% of compact bone (Clarke, 2008). It is known from studies in other species that compact and trabecular bones have different mineral turnover rates, for instance in humans, compact bone turnover is 0.25% (Clarke, 2008) and trabecular ~2% per month (Becker, 2001). In White Leghorn hens, bone mineral turnover rates are somewhat higher than in humans, with the turnover of compact bone of ~0.7% and of trabecular bone of ~4% per month, as determined by radio-isotope measurements for femur and tibiotarsus (Hurwitz, 1965).

Much of what is known about bone Pb dynamics is based on studies in mammals – including humans – and experimental studies with animal models (O'Flaherty, 1993; Inskip *et al.*, 1996; Fleming *et al.*, 1999). For example, differences in Pb accumulation in bone depending on compact and trabecular bone composition were found in humans and was attributed to differences in turnover rates of those bone types (Wittmers *et al.*, 1988). However, the avian skeleton is different than the skeleton of mammals, because in general birds have developed extremely lightweight skeletal systems that help aid in the generation of lift and thrust forces in flight, as well as helping them maintain flight over long periods of time (Kian, 2013). Moreover, in avian species, some bones are pneumatized, meaning the medullary cavity of the bone does not contain marrow (as in mammals) but rather membranous sacs of air to reduce weight (Cubo and Casinos, 2000). In condors, humerus and femur bones are pneumatized, whereas tibiotarsus is not and contains marrow. The presence of marrow is associated with increased blood perfusion, and since higher blood perfusion is associated with higher bone mineral turnover, differences in pneumatization of avian bones may also affect Pb accumulation in bone. These characteristics of avian bones may affect bone mineral metabolism and the toxicokinetics of Pb in bones, such that much of what has been determined in studies of mammalian skeletal Pb dynamics may not directly apply to condors.

No study in birds so far has tried to link bone Pb levels with Pb as a cause of mortality. However, there are a few studies indicating that bone Pb concentrations can be used as a marker for negative health outcomes in birds. For instance, an association

between bone Pb and a reduction of bone mineral density was found in a population of red-legged partridge (*Alectoris rufa*) (Álvarez-Lloret *et al.*, 2014), as well as in Egyptian vultures (*Neophron percnopterus*) (Gangoso *et al.*, 2009).

The main objective of this chapter is to determine the extent to which Pb concentrations vary within the skeleton of individual condors, and how skeletal Pb levels vary between condors with different Pb exposure histories. The overarching hypothesis I tested is that *Pb concentration in largely trabecular epiphyses will be higher in condors that died of Pb poisoning than in condors that died of other causes, whereas Pb concentration in compact bone of diaphyses will be higher in condors with a longer history of exposure than in condors with shorter or no history of exposure.* The ultimate goal of this research is to ascertain if bone Pb levels can be used to determine if Pb exposure contributed to the morbidity and mortality of condors that died in the wild.

2.2 Methods

2.2.1 Overall approach and sample selection

I started my analysis with an extensive sampling of the skeleton of a single bird (condor 553), and the results found for this condor were used to guide the selection of bones to be used in a larger sample of 11 birds with different Pb exposure history. The 11 birds were used to investigate the relationship between bone Pb concentration in three regions in three bones, totaling nine bone regions, and Pb exposure history.

Condor 553 was used to determine the extent to which Pb concentration differs within the condor skeleton, since the carcass of condor 553 was recovered from the

wild heavily scavenged and skeletonized; the state of the carcass precluded its use for other display options and allowed for extensive sampling of bone without the need for soft tissue dissection. I collected single or triplicate samples of bone from a wide array of bones with different structural and morphological characteristics, assuming that they would reflect differences in bone mineral metabolism and hence differences in Pb toxicokinetics. Specifically, triplicate samples were collected from proximal and distal epiphysis, proximal metaphysis and diaphysis of the femur, humerus, and tibiotarsus, fibula, vertebra (17^{th} lumbar), and sternum (base); single samples were collected from 3^{rd} cervical and 10^{th} thoracic vertebrae, and keel of sternum. Notably, there was evidence that condor 553 was highly exposed to Pb within 1 – 2 months prior to death, as evidenced by peak Pb concentration of ~10 μg/g (vs <0.5 μg/g background) in a growing flight feather collected and analyzed postmortem (Finkelstein unpublished data); this feather Pb level equates to an estimated blood Pb concentration of ~190 μg/dL (estimated from the segmented growing feather analysis, assuming feather growth of ~0.441 cm/day and the blood and feather Pb relationship of 19:1 (μg/dL:μg/g), as reported by (Finkelstein *et al.*, 2010)).

In order to explore how Pb levels differed across bones in condors with different Pb exposure histories, I selected 11 birds divided into three Pb exposure categories: 1) likely not exposed to elevated Pb (**Non-exposed**, n = 3, condors 445, 502, 639), 2) exposed to elevated Pb, but Pb poisoning was not the cause of death (**Other CoD**, n = 3, condors 301, 412, 630), and 3) Pb poisoning was the main CoD (**Pb CoD**, n = 5, condors 312, 345, 458, 478, 664). Birds in the Non-exposed category are birds that

either lived in captivity their whole life or were in the wild for less than a week before death and had a necropsy liver Pb concentration below the limit of detection, indicating that they were not exposed in the brief time that they were in the wild. Birds in the latter two Pb exposure categories were categorized based on a board-certified pathologist necropsy report. The description of condors used is summarized in Table 2.1. Condor 478 in the Pb CoD category was unique in this study since it was captured alive and treated for Pb toxicosis in the few days prior to its death, however due to dehydration a blood Pb measurement was not performed at this time. Chelation reduces the amount of Pb in blood and could also affect Pb concentration in bones.

Table 2.1 – Description of samples used in this study. Each sample is uniquely identified by a studbook number, which is also routinely used for monitoring during the lifetime of the bird. Each condor is classified into a Pb exposure category based on cause of death determined by a board-certified pathologist.

Studbook #	**Category / Cause of Death[i]**	**Sex**	**Age (years)**	**Time in the wild[ii]**	**Number of Chelation[iii] (past year/lifetime)**	**Liver Pb at necropsy (µg/g)[iv]**	**Most Recent Blood Pb (µg/dL)[v]**	**Date of most recent Blood Pb in relation to death[v]**
553	Suspected Pb	Male	2.7	9 months	NA	NA	NA	NA
445	Non-exposed	Male	2.9	0	0/0	NA	NA	NA
502	Non-exposed	Male	2.8	0	0/0	NA	NA	NA
639	Non-exposed	Female	2.5	3 days	0/0	< 1	NA	NA
301	Other CoD	Male	4	2.4 years	1/1	< 1	67	6 months prior
412	Other CoD	Male	5.7	4.4 years	0/2	< 1	32	1 year prior
630	Other CoD	Female	2.3	2.2 years	0/0	< 1	25	2 months prior
312	Pb CoD	Female	9.9	7.5 years	0/3	69	14	1 year prior
345	Pb CoD	Male	9	7.0 years	0/5	42	13	4 months prior
458	Pb CoD	Female	2.8	1.4 years	0/0	12	3	4 months prior
478[vi]	Pb CoD	Male	4	2.5 years	1/2	18	NA	NA
664	Pb CoD	Female	2.7	1.2 years	0/0	29	7	1.5 year prior

[i] Birds were divided into three categories: 1) Non-exposed (birds that lived only in captivity or a few days in the wild); 2) Other CoD (birds that lived in the wild and were likely elevated exposed to Pb, but the cause of death was not Pb); 3) Pb CoD (birds whose cause of death was Pb). Condor 553 did not have a definitive cause of death, but the pathologist indicates that Pb toxicosis is suspected.

[ii] Time in the wild is the time that a condor was free flying, based on release date for birds that hatched in captivity, hatch date for birds that hatched in the wild, and date of death. The days that a condor was captured and kept in captivity for observation or treatment were subtracted from the total time in the wild.

[iii] Chelation is a chemical treatment that helps eliminate trace metals from blood. Chelation treatment is applied to condors when blood Pb levels higher than 30 μg/dL are measured during routine monitoring (USFWS, 2014). I report the number of times chelation treatment was applied in the year prior to a condor's death and over the lifetime of a condor.

[iv] Liver Pb concentration is routinely measured during necropsy to inform the pathologist about the Pb poisoning status at the time of death.

[v] Blood Pb concentration is routinely measured biannually depending on a bird being captured by field teams. The latest available blood Pb concentration is reported here with respective date.

[vi] Condor 478 was unique in this study since it was captured alive and treated for Pb toxicosis in the few days prior to its death, however due to dehydration a blood Pb measurement was not performed at this time.

To assess the effect of Pb exposure in bone Pb concentration across the skeleton, I selected bones following the criteria:

- Bones that enable assessment of recent exposure and bones that enable the assessment of cumulative exposure, therefore, bones comprised mostly of trabecular (hypothesized to best reflect recent exposure) and bones that are comprised mostly of compact type bone (hypothesized to best reflect cumulative exposure);
- Bones that are readily available, i.e., bones that can be extracted from the extremities without significant damage to the carcasses, so as to preserve the carcass for possible display mounts.

Using the results from condor 553 and the fact that long bones have well-defined epiphysis (mostly trabecular) and diaphysis (mostly compact) and are usually found on the extremities, I determined that they were good candidates for the remaining of the study. Therefore, the bones selected were proximal and distal epiphysis and diaphysis of femur, humerus and tibiotarsus (Figure 2.1).

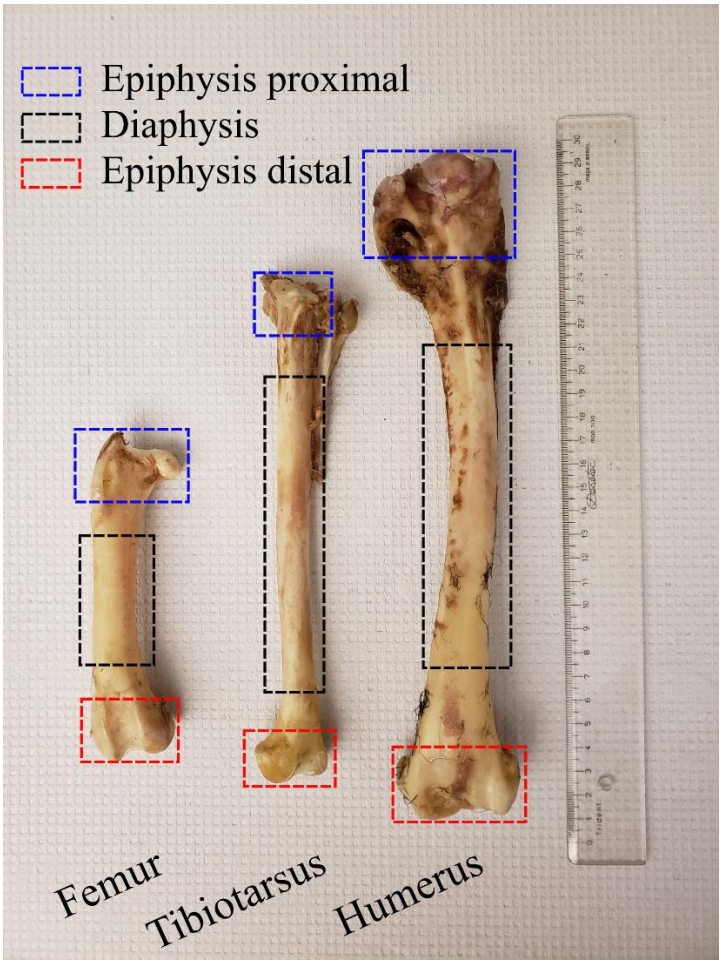

Figure 2.1 – Condor femur, tibiotarsus, and humerus. Proximal and distal epiphyses and diaphysis of each bone are marked in the figure.

2.2.2 Sample collection, processing and analysis

Bone samples were collected from the selected bones, and processed and analyzed in a high-efficiency particulate attenuated (HEPA) filtered air laboratory using established trace metal clean techniques, as described elsewhere (Smith, Osterloh and Flegal, 1996; Finkelstein, Gwiazda and Smith, 2003; Finkelstein *et al.*, 2010). Briefly, bone samples were collected using a titanium corer fitted to a power drill, or Dremel saw fitted with a stainless steel blade. Bone samples (50 – 100 mg) were

cleaned of adhering soft tissue with a stainless steel scalpel, rinsed with ultrapure grade water (Milli-Q system from Millipore, Inc), acetone and ultrapure 5% HNO_3 acid. Subsamples were then dried in an oven for 3 days (65° C) and weighed. Triplicates subsamples were used for each bone location. Bone samples were digested in 1 mL ultrapure concentrated HNO_3 in polytubes for 6 hours in a water bath (80°C); after digestion, samples were cooled for ~30 min. and 5 mL of ultrapure MQ water was added to dilute the samples.

Bone Pb concentrations were analyzed on quadrupole (Thermo XSeriesII) or magnetic sector inductively coupled plasma mass spectrometry (ICP-MS, Thermo Element XR magnetic sector), using ^{205}Tl as an internal standard. Between-run (i.e., long-term over several months) measurement precision was assessed using three samples and with RSD = ~15% (n = 5 runs), based on repeated measurements of bone samples. National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 1486 (Bone Meal) and 1400 (Bone Ash) were used to validate accuracy of the bone Pb measurements with recoveries of 81% – 99% (n = 15) for Bone Meal and 83% – 91% (n = 15) for Bone Ash. The analytical limit of detection for Pb measurements was 0.0047 ng/mL.

Calcium and phosphorus concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer Optima). National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 1486 (Bone Meal) and 1400 (Bone Ash) were used to validate accuracy of measurements, the recoveries for bone Ca measurements were of 93% – 105% (n = 15)

for Bone Meal and 92% – 108% (n = 15) for Bone Ash and of P measurements were of 88% – 116% (n = 15) for Bone Meal and 89% – 105% (n = 15) for Bone Ash. The analytical limit of detection for Ca measurements was 0.014 μg/mL and for P was 0.11 μg/mL.

2.2.3 Statistical analysis

Lead concentrations across the skeleton of condor 553 were compared with a Wilcoxon/Kruskal-Wallis test for bones that were sampled in triplicate. To determine whether the difference in Pb concentration across the skeleton can be influenced by the difference in mineral contents across the skeleton, I first tested whether calcium concentration varies across the skeleton of this condor using a Wilcoxon/Kruskal-Wallis test. Finally, I tested whether Pb:Ca ratio is statistically different than Pb concentration using a Wilcoxon signed rank test. All statistical tests for condor 553 were performed using measurements of each individual triplicate samples. I used averages and standard deviation of triplicates for each bone region to qualitatively compared differences in Pb levels across bones.

For the 11 condors in the three different Pb exposure history groups, triplicate values within a bone region were averaged before performing statistical tests, thus a particular bone region for each condor corresponds to a single data point in these analyses, bone Pb concentrations across bone regions were compared within each Pb exposure history group using a Wilcoxon/Kruskal-Wallis test. To compare Ca concentrations and P:Ca ratio, the 11 birds were considered together regardless of group classification. Calcium concentrations were compared using a

Wilcoxon/Kruskal-Wallis test. For parametric Pearson's correlation between Pb concentration between bone regions, log 10 transformation was used for normalization of data, using all 12 birds.

2.3 Results

2.3.1 Bone Pb concentration across a skeleton of a condor

Bone Pb concentration

In order to explore the extent that bone Pb levels differed across different bones within a condor, 18 different bones and bone regions were sampled, 15 in triplicate and three as individual samples. Condor 553, who was Pb exposed with an estimated peak blood Pb level of ~190 μg/dL several months before death, based on feather segment Pb analyses (Finkelstein, unpublished results). Bone Pb concentrations in condor 553 ranged between 10 and 125 μg/g (triplicate average) depending on bone and bone region (Figure 2.2). The ~2.5-fold range of Pb concentrations in diaphyses (10 – 25 μg/g), which are largely compact (e.g., ~95% in human femur (Clarke, 2008)), is much smaller than ~8-fold range in Pb concentrations in the largely trabecular bones (15 – 120 μg/g) (epiphyses, vertebrae, and sternum). The bone region with highest Pb concentration was tibiotarsus epiphysis proximal (~125 μg/g, average of triplicates) which was more than 2-fold higher than any other bone. The complete data set for condor 553 is listed in the Appendix A Table S.1.

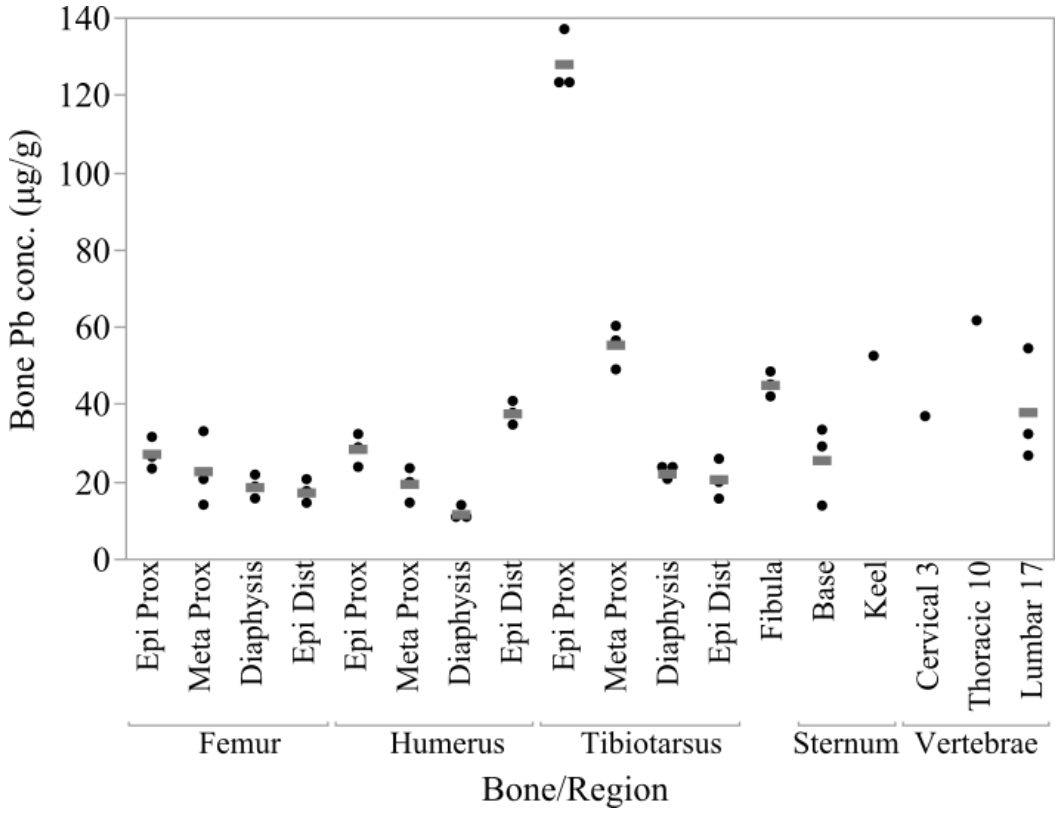

Figure 2.2 – Bone Pb concentrations across the skeleton of California Condor 553. Long bones (femur, humerus, and tibiotarsus) were analyzed in four distinct regions: proximal epiphysis (Epi Prox), proximal metaphysis (Meta Prox), diaphysis, and distal epiphysis (Epi Dist). For sternum both base and keel were analyzed independently, also three vertebrae (3rd cervical, 10th thoracic and 17th lumbar) were analyzed independently. For each bone region, dots represent a single replicate measurement, the dash represents the average of the triplicates. Keel, 3rd cervical and 10th thoracic were analyzed as a single sample. The Pb concentration is significantly different across 17th vertebra, base of sternum, fibula, proximal and distal epiphyses of femur, humerus and tibiotarsus (Wilcoxon/Kruskal-Wallis, P = 0.0001, ChiSquare = 49.5, DF = 14).

In order to determine whether bone Pb concentrations differed across the skeleton of condor 553, a Wilcoxon/Kruskal-Wallis non-parametric statistical test was performed and revealed that the Pb concentrations were statistically different across 17th lumbar vertebra, base of sternum, fibula, proximal and distal epiphysis, proximal metaphysis and diaphysis of femur, humerus and tibiotarsus of this condor (P < 0.0001, ChiSquare = 49.5, DF = 14). Samples from mostly trabecular bone (epiphyses of femur,

humerus, and tibiotarsus, vertebra and sternum) had up to 10-fold higher Pb concentrations than samples from mostly compact bone (diaphysis of femur, humerus, and tibiotarsus), this was confirmed by a Mann–Whitney U test performed over the averages of triplicates for each bone region ($P < 0.0004$, ChiSquare = 12.8, DF = 1).

Bone mineral content

To determine whether the differences in bone Pb concentrations was influenced by or associated with differences in bone mineral content, I analyzed bone Ca concentrations. The results showed that bone Ca concentrations across the 18 bones and bone regions sampled ranged between 150 and 260 mg/g in condor 553 (Figure 2.3). A Wilcoxon/Kruskal-Wallis non-parametric test revealed that Ca concentrations were statistically different across 17th lumbar vertebra, base of sternum, fibula, proximal and distal epiphysis, proximal metaphysis and diaphysis of femur, humerus and tibiotarsus of this condor ($P = 0.0005$, ChiSquare = 38.1, DF = 14) (Figure 2.3A). Tibiotarsus epiphysis proximal had the lowest Ca concentration among the long bones analyzed, with average of ~170 mg/g (Figure 2.3A).

Since bone Ca concentrations differed across the skeleton of 553, I explored whether the differences in Ca concentrations was reflective of variation in bone mineral content by investigating P:Ca atom ratio in each sample. As expected, Ca and P concentrations were highly correlated statistically (Pearson correlation $R = 0.974$, $P < 0.0001$, DF = 47). Moreover, the P:Ca atom ratio observed from the linear regression for this condor was 0.58 mol P/ mol Ca (Figure 2.3B), which is within the expected range ~0.53 – 0.64 mol P/mol Ca (Singh et al. 2007).

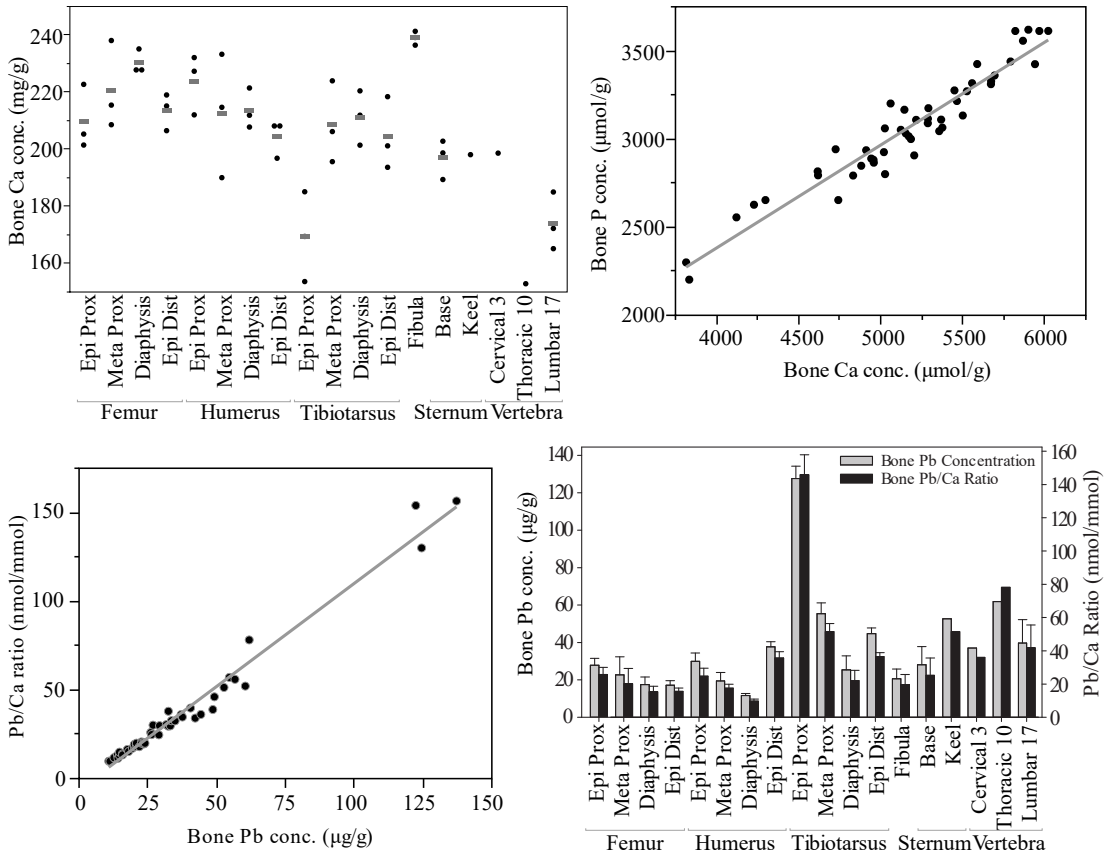

Figure 2.3 – (A) Bone Ca concentration; (B) Bone P versus Ca concentration; (C) Bone Pb concentration versus Pb:Ca atom ratio; and (D) Bone Pb concentration and Pb:Ca ratio across the skeleton of condor 553. Long bones (femur, humerus, and tibiotarsus) were analyzed in four distinct regions: proximal epiphysis (Epi Prox), proximal metaphysis (Meta Prox), diaphysis, and distal epiphysis (Epi Dist); for sternum both base and keel were analyzed independently, also three vertebrae (3rd cervical, 10th thoracic and 17th lumbar) were analyzed independently. Keel, 3rd cervical and 10th thoracic were analyzed as a single sample. In Figure 2.3A, for each bone region, dots represent a single replicate measurement, the dash represents the average of the triplicates. The Ca concentration is significantly different across 17th vertebra, base of sternum, fibula, proximal and distal epiphyses of femur, humerus and tibiotarsus (Wilcoxon/Kruskal-Wallis, P = 0.0005, ChiSquare = 38.1, DF = 14). In Figure 2.3B and C, each dot represents the measurement for each replicate (n = 3). Ca and P concentrations were statistically correlated (Pearson correlation R = 0.974, P < 0.0001, DF = 47) and Pb concentration and Pb:Ca ratio were also statistically correlated (Pearson correlation R = 0.984, P < 0.0001, DF = 47). In Figure 2.3D, each bar represents the average ± SD of the replicates. The Pb:Ca ratio was not significantly

different than the Pb concentration for each bone region (Wilcoxon signed rank test, P = 0.999, S = 301, DF = 47).

Pb:Ca ratio

Finally, to determine whether the variation in Pb concentrations across different bones and bone regions in condor 553 could be explained by differences in bone Ca content (as a measure of bone mineral), I explored whether the bone Pb concentrations were statistically correlated with the Pb:Ca ratio. Results showed that they were highly correlated (Pearson correlation R = 0.984, P < 0.0001, DF = 47) (Figure 2.3C). I also performed a paired Wilcoxon signed rank test to determine whether the Pb:Ca atom ratio, expressed in nmol/mmol, in different bones was different than the Pb concentration, expressed in μg/g, for each bone region. The unit scales were selected so that the absolute numbers were comparable in range. I found that there was no significant difference between Pb:Ca atom ratio and Pb concentration across 17th lumbar vertebra, base of sternum, fibula, proximal and distal epiphysis, proximal metaphysis and diaphysis of femur, humerus and tibiotarsus of this condor (P = 0.999, S = 301, DF = 47) (Figure 2.3D). Since Pb content in bone still differed even when normalizing for Ca, Pb variation across the skeleton could not be attributed to bone mineral variation across the skeleton.

2.3.2 Bone Pb concentration in the skeleton of 11 condors with different Pb exposure histories

Bone Pb concentration

To understand how bone Pb concentrations differed across the proximal and distal epiphyses and diaphysis of femur, humerus, and tibiotarsus in birds with different Pb exposure histories, I selected 11 condors grouped into three categories of Pb exposure history: 1) **Non-exposed**, or birds with no known history of Pb exposure (n = 3); 2) **Other CoD**, or birds with a history of Pb exposure but whose cause of death was something other than Pb poisoning (n = 3); and 3) **Pb CoD**, or birds with a history of Pb exposure and whose cause of death was Pb poisoning (n = 5). The complete data set for the 11 condors is listed in the Appendix A Table S.1. For the Non-exposed condors, the bone Pb levels were consistently very low, between 0.058 and 0.860 µg/g, and relatively invariant within a bird (Figure 2.4A). In contrast, bone Pb levels in birds with a history of Pb exposure (Other CoD and Pb CoD, range ~10 – 200 µg/g) are >100-fold higher than the Non-exposed birds (Figure 2.4). A Wilcoxon/Kruskal-Wallis test was performed to compare each bone region across bones within each category. No significant difference was observed across bone regions in the Non-exposed and Other CoD groups. For the Pb CoD, there was statistical significance when comparing Pb concentration in proximal epiphysis across humerus femur and tibiotarsus (Wilcoxon/Kruskal-Wallis, P = 0.0305, ChiSquare = 6.98, DF = 2) and in distal epiphysis across humerus, femur and tibiotarsus (Wilcoxon/Kruskal-Wallis, P = 0.0183, ChiSquare = 8.00, DF = 2).

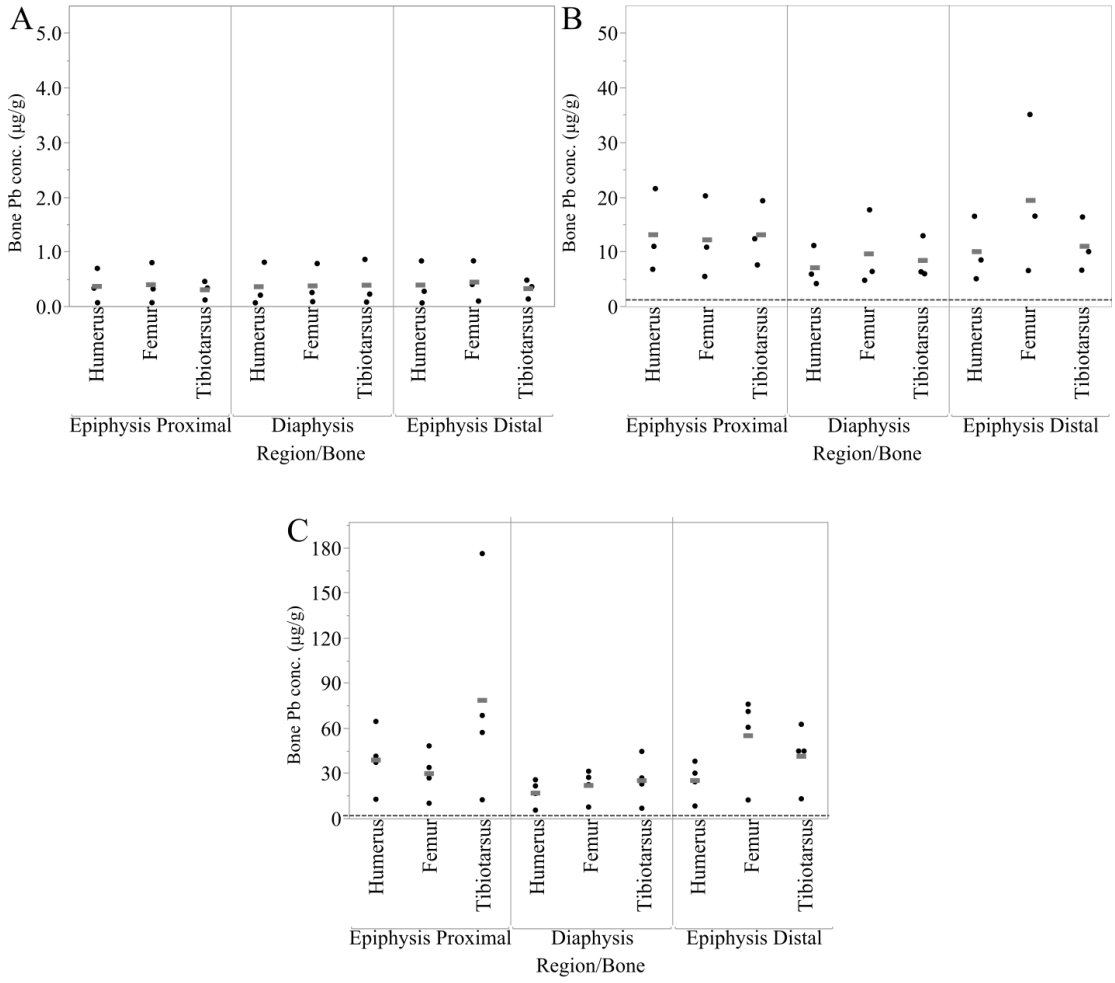

Figure 2.4 – Bone Pb concentration in proximal and distal epiphyses and diaphysis of three long bones (humerus, femur, and tibiotarsus), split into three Pb exposure categories according to the bird classification criteria from the Methods section and Table 2.1: (A) likely not elevated exposed to Pb (Non-exposed, n = 3); (B) Elevated exposed to Pb, but Pb not CoD (Other CoD, n = 3); and (C) Pb diagnosed as CoD (Pb CoD, n = 5). Each dot represents the average of triplicates for a single bird, and the dash represents the average across birds. Wilcoxon/Kruskal-Wallis was performed to compare each bone region across bones within each category. No significant difference was observed across bone regions in the Non-exposed and Other CoD groups. For the Pb CoD, there was statistical significance when comparing Pb concentration in proximal epiphysis across humerus femur and tibiotarsus (Wilcoxon/Kruskal-Wallis,

P = 0.031, ChiSquare = 6.98, DF = 2) and in distal epiphysis across humerus, femur and tibiotarsus (Wilcoxon/Kruskal-Wallis, P = 0.018, ChiSquare = 8.00, DF = 2).

An accurate biomarker of Pb exposure should reflect the unique Pb exposure history of each individual, therefore, good Pb exposure biomarkers have variability across condors that reflect different Pb exposure history. Thus, I looked into variability of bone Pb concentration in each bone region within a group. For condors in the Other CoD category, the femur epiphysis distal had high variability (RSD = 65% across birds) in Pb concentration (Figure 2.4B), which is higher than the variability presented by, for instance humerus diaphysis (RSD = 44% across birds) and tibiotarsus diaphysis (RSD = 40% across birds), with one condor (412) having 2-fold higher Pb concentration (~30 μg/g versus ~15 μg/g) in this bone region than the other two birds in this category. Also, bone Pb levels had a 3 – 13-fold range between bone regions in exposed condors (Other CoD and Pb CoD categories, Figure 2.4B-C), and ~2-fold range in unexposed birds (Non-exposed category, Figure 2.4A). For birds in the Pb CoD category, Pb concentrations across proximal epiphysis largely differed between humerus, femur and tibiotarsus, with humerus ranging between 13 and 65 μg/g (RSD = 57%), femur ranging between 10 and 50 μg/g (RSD = 54%), and tibiotarsus ranging between 13 and 170 μg/g (RSD = 80%). Bone Pb concentrations also had high variability across the distal epiphysis, with humerus ranging between 9 and 39 μg/g (RSD = 47%), femur ranging between 13 and 77 μg/g (RSD = 63%) and tibiotarsus ranging between 14 and 64 μg/g (RSD = 47%). Tibiotarsus epiphysis proximal presented the highest variability across birds in the Pb CoD group, ranging from 13 to 170 μg/g (RSD = 80%) among all bone regions (Figure 2.4C). In each of the categories, Pb concentrations in diaphyses did not

differ across bones (Figure 2.4). The Pb concentrations in epiphyses were ~2.8-fold on average higher than diaphysis of each bone in the Pb CoD group (Figure 2.4C) while only ~2-fold in the Other CoD group (Figure 2.4B).

I also analyzed the inter-bone region correlation of Pb concentrations for all condors used in this study (n = 12) using pairwise Pearson's correlation (proximal and distal epiphyses and diaphysis of humerus, femur and tibiotarsus). All pairs of bones/bone regions were highly correlated (Pearson's correlation, R between 0.947 and 0.994, P's between < 0.0001 and 0.0015) (Table 2.2).

Table 2.2 – Pearson correlation between multiple bones and bone regions of all condors used in this study, using log 10 transformed data for normalization. First line in each cell is the R, and the second line is the P value statistical significance, n = 12 birds, using average of triplicates per bone region.

	Femur diaphysis	Femur epiphysis distal	Femur epiphysis proximal	Humerus diaphysis	Humerus epiphysis distal	Humerus epiphysis proximal	Tibiotarsus diaphysis	Tibiotarsus epiphysis distal
Femur epiphysis distal	0.986 (<.0001)							
Femur epiphysis proximal	0.969 (0.0005)	0.987 (<.0001)						
Humerus diaphysis	0.992 (<.0001)	0.990 (<.0001)	0.978 (0.0002)					
Humerus epiphysis distal	0.987 (<.0001)	0.982 (<.0001)	0.973 (0.0003)	0.994 (<.0001)				
Humerus epiphysis proximal	0.979 (0.0002)	0.986 (<.0001)	0.993 (<.0001)	0.989 (<.0001)	0.981 (0.0001)			
Tibiotarsus diaphysis	0.969 (0.0005)	0.969 (0.0005)	0.976 (0.0002)	0.985 (<.0001)	0.973 (0.0004)	0.990 (<.0001)		
Tibiotarsus epiphysis distal	0.970 (0.0005)	0.967 (0.0006)	0.967 (0.0006)	0.983 (<.0001)	0.987 (<.0001)	0.980 (0.0001)	0.974 (0.0003)	
Tibiotarsus epiphysis proximal	0.947 (0.0025)	0.955 (0.0015)	0.970 (0.0004)	0.974 (0.0003)	0.976 (0.0002)	0.980 (0.0001)	0.984 (<.0001)	0.987 (<.0001)

Bone mineral contents and Pb:Ca ratio

As was observed for condor 553, Ca concentrations differed between and within bones of condors, based on analyses of bones from all 12 birds (range: 100 – 300 mg/g) (Figure 2.5A). Furthermore, P and Ca were highly correlated, as expected (Pearson correlation $R = 0.969$, $P < 0.0001$, $DF = 296$) and the molar ratio between P and Ca was ~0.514 mol P/mol Ca) (Figure 2.5B), again similar to expected value based on the ratio of Ca and P in hydroxyapatite mineral (Singh *et al.*, 2007). To determine whether the differences in Pb concentrations between bones within a single bird was associated with differences in bone mineral content, I performed a paired Wilcoxon signed rank test to determine whether the Pb:Ca atom ratio (in nmol/mmol) was different than the Pb concentration (in μg/g) for each bone region. The unit scales were selected so that the absolute numbers were comparable in range. I found that there was no significant difference between Pb:Ca atom ratio and Pb concentration across proximal and distal epiphysis, and diaphysis of femur, humerus and tibiotarsus (Wilcoxon signed rank test, $P = 1.00$, $S = 12100$, $DF = 323$).

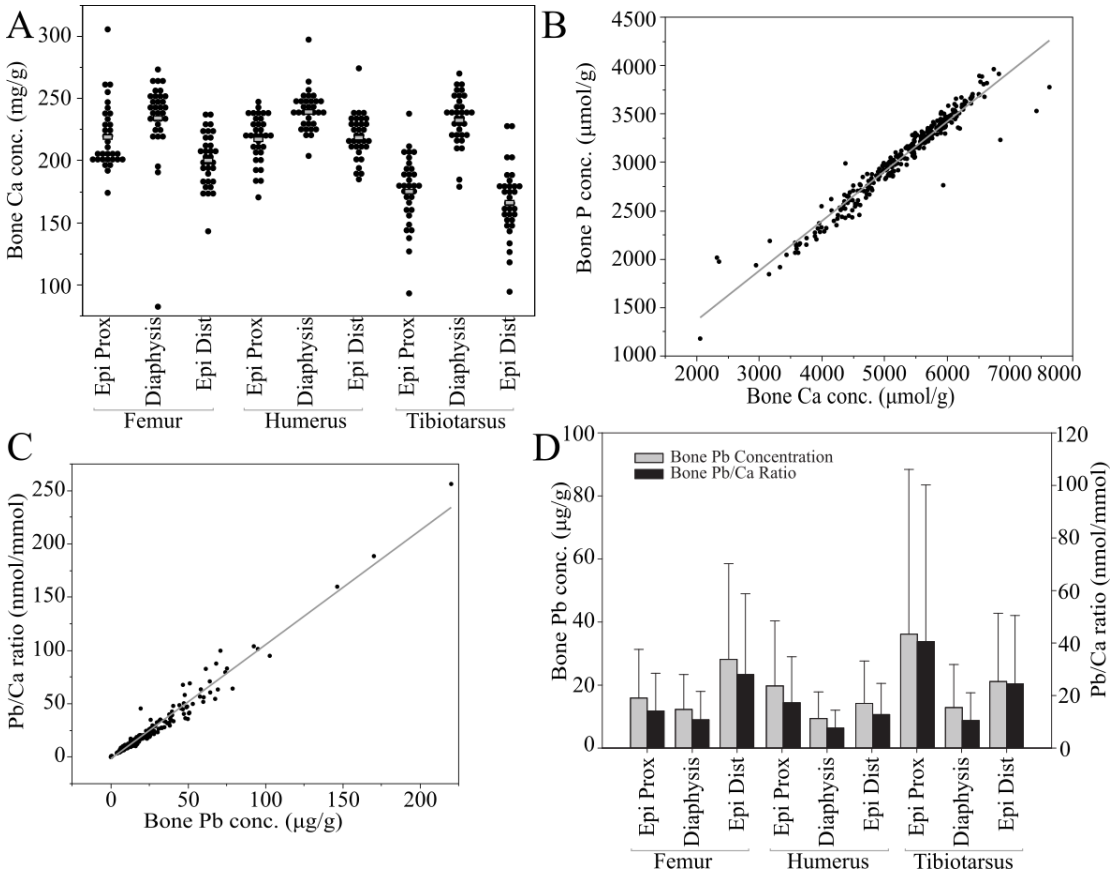

Figure 2.5 – (A) Bone Ca concentration; (B) Bone P versus Ca concentration; (C) Bone Pb concentration vs Pb:Ca ratio; and (D) Bone Pb concentration and Pb:Ca ratio across the skeleton of ten California condors. Long bones (femur, humerus, and tibiotarsus) were analyzed in three distinct regions: proximal epiphysis (Epi Prox), diaphysis, and distal epiphysis (Epi Dist). In Figure 2.5A, for each bone region, dots represent a single replicate measurement, the dash represents the average of the triplicates. The Ca concentration is significantly different across bone regions (Wilcoxon/Kruskal-Wallis, $P < 0.0001$, ChiSquare = 172, DF = 8). In Figure 2.5B and C, each dot represents the measurement for a single replicate. Ca and P concentrations were statistically correlated (Pearson correlation $R = 0.969$, $P < 0.0001$, DF = 323) and Pb concentration and Pb:Ca ratio were also statistically correlated (Pearson correlation $R = 0.984$, $P < 0.0001$, DF = 323). In Figure 2.5D, each bar represents the average ± SD of the replicates. The Pb:Ca ratio was not significantly different than the Pb concentration for each bone region (Wilcoxon signed rank test, $P = 1.000$, $S = 12100$, DF = 323). This

confirms the findings for condor 553, Ca varies across bones and this variation is not sufficient to explain the Pb concentration variation across bones.

2.4 Discussion

The main objective of this chapter was to determine the extent that Pb concentrations differed across multiple bones and bone regions within the condor skeleton, and how skeletal Pb levels differed between bones and between birds with different Pb exposure histories. First, I explored Pb concentrations across the skeleton of a single condor (553) that had a known history of Pb exposure prior to death; the carcass of this condor was also heavily skeletonized. Then, based on the results from this condor, I selected proximal and distal epiphyses and diaphysis of humerus, femur, and tibiotarsus to expand my analyses to 11 condors with different Pb exposure histories to explore how Pb exposure history affected Pb levels across different bones within a bird. The ultimate goal of this research is to inform whether condor bone Pb levels can be used to assess if Pb contributed to a bird's morbidity/mortality and thus provide a tool that allows to assess whether a condor was Pb poisoned at the time of death when soft tissues are not available.

Currently, Pb exposure history is assessed in California Condors through annual or biannual blood Pb monitoring, but since the half-life of Pb in condor's blood is about 13 days (Fry and Maurer, 2003), it may capture only ~4 – 10% of a condor's annual exposure history (Finkelstein *et al.*, 2010). Furthermore, given the brevity of elevated Pb concentrations in blood, it is unlikely to capture the peak magnitude of an exposure event (Finkelstein *et al.*, 2012). Blood Pb concentrations are meaningful as real time measurements in an individual condor, especially for clinical management of Pb

poisoning. However, when considering long-term Pb exposure or studying trends in an individual condor or a given population, morbidity and mortality risk is likely better measured by a bird's comprehensive Pb exposure history (both cumulative and acute), as it has been shown in humans (Hu *et al.*, 1994, 1996; Cheng *et al.*, 2001). Moreover, the lack of an accurate Pb exposure biomarker that reflects a condor's exposure history makes it hard to assess the degree to which Pb poisoning impacts condor population health. In addition to the afore mentioned objective, this work is also a first step towards understanding which bones may be useful in assessing cumulative Pb exposure in condors.

2.4.1 Bone Pb concentration across a skeleton of a condor

To better understand Pb exposure history of condor 553 and analyze the results in a more informed manner, I first looked into Pb concentration from sequential feather analysis and other known history that is relevant to Pb exposure. The analysis of Pb in sequential feather segments has been an useful tool to evaluate a condor's Pb exposure history over a period of several months (Finkelstein *et al.*, 2010). The levels of Pb in blood of condor 553, as estimated by segmented feather analysis, were over 100 μg/dL in the few weeks before its death, peaking at 350 μg/dL around a month prior to death (Finkelstein, unpublished data). From 2 – 4 months prior to death, feather Pb levels were ~0.5 μg/g (estimated blood Pb ~10 μg/dL). The feather analysis covers a total of four months out of the ten months in which this condor was in the wild. Although a definitive cause of death was not determined for this condor, estimated by feather analysis, the estimated elevated blood Pb concentrations were above 50 μg/dL, which

are usually considered indicative of Pb toxicosis (de Francisco, Ruiz Troya and Agüera, 2003; Rideout *et al.*, 2012). This condor was two and a half years old and lived in the wild for 10 months, and thus presumably did not have a long history of cumulative Pb exposure, however, the feather analysis supports that this condor was highly exposed to Pb in the month prior to death.

For condor 553, bone Pb concentrations (Figure 2.2) are statistically different across the bones that were sampled in triplicate (17^{th} vertebra, base of sternum, fibula, proximal and distal epiphysis, proximal metaphysis and diaphysis of femur, humerus and tibiotarsus). Of note, bone regions that are mostly trabecular (epiphyses, metaphysis, sternum and vertebrae) having statistically higher Pb concentrations than regions that are largely compact (diaphysis). In combination with the fact that this condor was exposed to Pb in the month prior to death, the fact that largely trabecular bone (epiphyses, vertebrae and sternum) had higher Pb concentration relative to compact bone is consistent with the expectation that largely trabecular bones and bone regions have a faster Pb uptake during an event of Pb exposure (O'Flaherty, 1993).

The high Pb concentration in tibiotarsus epiphysis proximal in condor 553, compared to epiphyses of humerus and femur in this condor (Figure 2.2), suggests that the bone mineral turnover, and thus bone Pb turnover in the tibiotarsus epiphysis proximal is higher than that of epiphyses in humerus and femur. A possible contributing factor for the difference in Pb concentration between tibiotarsus and other bones is that femur and humerus are pneumatized bones in condors, whereas the tibiotarsus is non-pneumatized. Pneumatization of bones in avian species is the development of air filled

cavities in lieu of bone marrow within a bone (Cubo and Casinos, 2000). Pneumatization is known to reduce the amount of vascularization (Beaumont, 1968) and thus of bone perfusion. Since Pb is distributed through the body by blood and that bone mineral turnover increases with increased perfusion (O'Flaherty, 1993), the higher bone Pb levels in the tibiotarsus epiphysis proximal versus the humerus and femur epiphysis proximal is consistent with a higher bone mineral turnover and Pb accumulation rates with exposure in the non-pneumatized tibiotarsus following 553's recent Pb exposure. However, pneumatization cannot explain differences in bone Pb concentration within tibiotarsus, in which case differences are likely attributed to differences in the relative trabecular and compact composition of each bone region.

I also investigated whether the variation in Pb concentrations across bones is influenced by differences in mineral content across the skeleton (Figure 2.3A). Bone Ca was higher in compact than in trabecular bone, which is expected form evidence in White Leghorns (Jendral *et al.*, 2008). Since Pb substitutes for Ca in the hydroxyapatite mineral (Barton, 1984), differences in the bone mineral density (i.e., concentration of Ca per mg of bone) in a bone could influence the bone Pb concentration. First I established that bone Ca concentration was a reasonable surrogate of bone mineral content by showing that bone P was highly correlated with bone Ca with a linear regression slope of 0.58 mol of P/ mol of Ca (Pearson correlation $R = 0.974$, $P < 0.0001$, DF = 47) (Figure 2.3B), and that this P:Ca relationship is comparable with hydroxyapatite mineral values in the literature (~0.53 – 0.64 mol P/mol Ca) (Singh *et al.*, 2007).

Then, I explored whether bone Ca concentration, as a surrogate of bone mineral content, could explain some of the variation in Pb concentration across different bones. I found that, while the Ca concentration ranged from 150 – 260 mg/g across bones of 553, the Pb:Ca molar ratio was highly correlated with the Pb concentrations (Pearson's correlation $R = 0.984$, $P < 0.0001$, $DF = 47$) (Figure 2.3D). Moreover, I performed a paired t-test between the Pb concentration versus the Pb:Ca molar ratio of each sample (after adjusting the units for the Pb:Ca molar ratio to nmol Pb/mmol Ca to yield a numerical scale similar to the bone Pb concentrations) (Figure 2.3D) and found there was no statistical difference between them ($T(47) = 0.378$, $P = 0.670$). The lack of a significant effect of bone Ca content on bone Pb concentration is not unexpected here, since bone Pb concentrations varied over 14-fold between bone samples in condor 553, whereas bone Ca concentrations varied only 1.5-fold across the skeleton of this condor. Therefore, I concluded that the difference in Pb concentration between bones and bone regions cannot be simply attributed to differences in bone mineral content across bone region.

Although the majority (>90% (Bergdahl *et al.*, 1998)) of body Pb resides within the skeleton of vertebrates and Pb is known to accumulate within bone over time, the toxicokinetics of Pb uptake into bone is typically slower than in soft tissues such as the liver and kidney (O'Flaherty, 1993). Thus, birds acutely exposed to Pb may acquire a highly elevated soft tissue Pb burden and die before significant amounts of Pb have been incorporated into bone (Pain *et al.*, 2005). In bald eagles (*Haliaeetus leucocephalus*), there have been reports of birds experimentally exposed to Pb shot,

dying with liver Pb concentrations of 17 μg/g (i.e., 17-fold higher than background liver Pb levels of <1 μg/g), but had relatively lower levels of Pb (~10 μg/g) in femur, humerus and tibiotarsus (region not specified) (Pattee *et al.*, 1981), which is considered by some to be the threshold for abnormal Pb exposure in birds of prey (Mateo, Taggart and Meharg, 2003). In waterfowl, bone Pb concentrations of >20 μg/g are associated with excessive exposure (Franson, 1996). My data show that Pb exposed condors (Other CoD and Pb CoD groups), had bone Pb levels of as low as 5 μg/g (average 29 μg/g) which is lower than levels previously reported in the literature as indicative of Pb exposure in raptors (Pattee *et al.*, 1981; Mateo, Taggart and Meharg, 2003). When examining bone Pb concentrations from condor 553 (Figure 2.2), it is clear that it is not possible to define a specific bone Pb concentration as an indicator of Pb poisoning that can be widely applied to any bone. For instance, tibiotarsus epiphysis proximal in this bird is well above 150 μg/g, which would qualify it as Pb poisoned, however, if the bone used were diaphysis of humerus, and using the 10 μg/g proposed above, one could have concluded that this bird was not poisoned, which is clearly not the case. Therefore, to fully interpret bone Pb concentration, it is necessary to take into account which bone and bone region was analyzed. Moreover, since various species present pneumatization in different bones, the levels considered for one species may not apply to others.

2.4.2 Bone Pb concentration in the skeleton of 11 condors with different Pb exposure histories

Bone Pb levels may better reflect cumulative Pb exposure history than blood Pb concentration while also helping to inform lead exposure status at time of death. In

humans, a strong association (R = 0.889) was found between tibia Pb concentration and integral of blood Pb concentration (Bergdahl *et al.*, 1998), which indicates that bone Pb reflects cumulative Pb exposure history. Raptors accumulated ~40 μg/g of Pb in humerus when dosed with 3 mg of Pb/kg body weight/day for 30 weeks (Mateo, Taggart and Meharg, 2003), consistent with evidence showing that bone Pb is an indicator of cumulative Pb exposure (Wittmers *et al.*, 1988; Bergdahl *et al.*, 1998). Moreover, there is evidence that bone Pb may be a better indicator of cumulative Pb exposure that blood Pb or liver Pb in birds. For instance, American kestrels fed with mice containing one Pb shot for 60 days followed by feeding mice without Pb shot 15 days immediately before euthanasia, accumulated around 30 μg/g Pb in tibiotarsus but only 0.37 μg/g in liver (Stendell, 1980). There is also evidence that bone Pb concentration can be an accurate biomarker of acute exposure and mortality by Pb toxicosis. For instance, a significant correlation between Pb concentration in liver and humerus ($R = 0.581$, $P < 0.005$) and femur ($R = 0.678$, $P < 0.001$) was found in wild white-headed ducks (Mateo *et al.*, 2001). Also, Pb poisoning was suggested to be a contributing factor to the death of an Eurasian eagle-owl that had 185 μg/g of Pb in its bone (Mateo, Taggart and Meharg, 2003). Collectively, this evidence suggests that bone Pb concentration is a good predictor of the complex and dynamic exposure history and Pb mortality risk, both as a surrogate for time-integral Pb exposure over the course of years (Hu, Rabinowitz and Smith, 1998) and as a surrogate for acute exposure. Given the lower mineral turnover in compact bone than in trabecular bone, and the relatively low age of condors analyzed in my study (2 to 9 years), it is somewhat expected that

Pb concentration in diaphysis of femur, humerus and tibiotarsus is lower than that of epiphyses of those bones, since the slower turnover of diaphyses makes those regions more reflective of cumulative Pb exposure.

Background bone Pb concentrations in birds are not well-defined and there is no consensus about a specific threshold value that defines background versus elevated. Natural levels of exposure no longer exist due to anthropogenic contamination of the biosphere with Pb (Patterson, Shirahata and Ericson, 1987). Bone Pb levels for condors in the Non-exposed category were similar between different bones within a single bird but differed between the individual Non-exposed birds, i.e., bone Pb concentration was (average ± SD) for condor 445, 0.09 ± 0.03 μg/g; for condor 502, 0.30 ± 0.07 μg/g; and for condor 639, 0.72 ± 0.16 μg/g (Figure 2.5A). Bone Pb levels varied 10-fold between these three birds that were only exposed to background levels of Pb, but all three condors had bone Pb concentration <1 μg/g, suggesting that this is the background bone Pb levels for California condors, living in California around the 2000s.

For the five condors in the Pb CoD group, the high liver Pb concentrations found at necropsy (12 – 59 μg/g) is evidence of very elevated Pb exposures in the few weeks prior to death. Given that Pb CoD condors were exposed to Pb in the few weeks prior to death, the high Pb concentration in tibiotarsus epiphysis proximal (on average ~2.7-fold higher than proximal epiphyses of femur and humerus) (Figure 2.4C) suggests that Pb incorporated relatively quickly into tibiotarsus epiphysis proximal. The higher Pb incorporation in tibiotarsus epiphysis proximal suggests that the mineral, and thus, Pb turnover, in this bone region is higher than in other bone regions. This

result is compatible with the findings that epiphysis, comprised of mainly trabecular bone, has higher mineral turnover than diaphysis, which are comprised mainly of compact bone. For instance, in White Leghorn hens, the turnover of trabecular bone is ~4% and only ~0.7% in compact bone per month (Hurwitz, 1965) – but also with the fact that, in condors, tibiotarsus is a non-pneumatized bone, which is may increase perfusion and mineral turnover rate, whereas humerus and femur are pneumatized bones in condors. Therefore, the differences in bone Pb concentration across the skeleton are a combination of two factors: differences in trabecular to compact composition in each bone region (within a bone) and pneumatization (between bones).

The high variability in bone Pb concentrations across birds (Figure 2.4) for the same bone region – for instance 65% RSD for femur epiphysis distal in Other CoD birds, and 95% RSD for tibiotarsus epiphysis proximal in Pb CoD birds – seems to be related to each individual bird's unique Pb exposure history. The Pb concentrations across bones and bone regions by Pb exposure category, presented in Figure 2.4, suggests that the magnitude of Pb exposure inferred from bone would differ substantially depending on which bone and/or bone region is selected as an indicator of Pb exposure. If a single bone Pb concentration were to be established as indicative of Pb exposure, choosing different bones would Pb to different outcomes in terms of whether each bird was poisoned or not at the time of death. Moreover, the bone selected as indicative of Pb exposure should have a wide Pb concentration range between condors that died of Pb poisoned and condors that died of other causes. The data presented – where tibiotarsus epiphysis proximal has the highest Pb concentration in

the Pb CoD category – suggests that Pb concentration in tibiotarsus epiphysis proximal seems to be the most fitting sample to measure recent Pb exposure.

From the analysis in Table 2.2, there is high correlation between bone Pb concentration between bones and bone regions, however, from the results in Figure 2.4, it seems that some bone regions appear to be more sensitive to Pb uptake. For instance, tibiotarsus epiphysis proximal and femur epiphysis distal, which had high variability due to presumed differences in individual Pb exposure history. Moreover, since Pb concentrations differ across bones and bone regions (Figure 2.4), studies using bone Pb to assess Pb exposure need to be consistent in the choice of bone and bone region. Existing studies with other avian species commonly use tibiotarsus (Hontelez, Dungen and Baars, 1992; García-Fernández *et al.*, 1997; Janiga and Žemberyová, 1998) or femur (Wayland, Neugebauer and Bollinger, 1999; Eeva *et al.*, 2000; Mateo, Taggart and Meharg, 2003; Pain *et al.*, 2005; Svanberg *et al.*, 2006; Álvarez-Lloret *et al.*, 2014).

Finally, I also confirmed that calcium concentrations found across bones and bone regions analyzed for condor 553 was comparable with the set of 11 condors. Calcium, and thus, the mineral contents of bone, differed between and within bones and the differences found did not prove sufficient to account for differences in Pb concentration, corroborating the results from 553 that differences in bone mineral content (as indicated by Ca concentration) does not explain the differences in bone Pb.

2.5 Conclusion

The main finding presented in this chapter is that Pb concentrations vary within the condor skeleton. The differences in Pb concentration between bones of a single bird

could partially be attributed to differences in bone mineral turnover in each bone region. I also observed that those differences in bone Pb concentrations in different bones cannot be explained by differences in bone mineral content, as indicated by differences in calcium concentration in each bone and bone region. Regions of bone with faster turnover rates, such as largely trabecular bones, are more sensitive to Pb uptake, which leads to a more rapid uptake of Pb in exposure events. On the other hand, bones with slower turnover rates, such as mostly compact bones, e.g., diaphysis of long bones, appear to be less sensitive to individual events of exposure and reflect the accumulation of effects over longer periods of time. One consequence of this finding is that the magnitude of Pb exposure inferred from bone would differ substantially depending on which bone and/or bone region is selected as an indicator of Pb exposure.

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Chapter 3) Improving lead exposure diagnosis using bone lead concentrations in California Condors

3.1 Introduction

Lead (Pb) poisoning is the primary threat to the recovery of the endangered California condor (Finkelstein *et al.*, 2012). However, the full impact of Pb poisoning on condor mortality is likely underestimated because it is not always possible to determine a definitive cause of death (CoD). Currently, the gold standard to diagnose whether a condor died of Pb toxicosis is blood and liver Pb concentrations (Rideout *et al.*, 2012), which is only possible when condor carcasses are recovered fresh from the wild with those tissues available. Carcasses found heavily scavenged often have most of the soft tissue missing and only bone and feathers are recovered. Out of 82 adult condors recovered from the wild between 1996 and 2010, 23 (28%) died of Pb toxicosis and for 20 (24%) condors it was not possible to diagnose a cause of death due to the carcass being scavenged, too autolyzed for evaluation, or when evidence pointing to a particular cause could not be identified or was only speculative (Rideout *et al.*, 2012). These findings suggest that a larger proportion than 28% may have died of Pb toxicosis.

In humans, bone Pb is known to be a good biomarker of chronic, lifelong Pb exposure and Pb toxicity, since Pb accumulates in bone over periods of weeks to years (Hu, 1998; Barbosa *et al.*, 2005). Moreover, lead accumulates at different rates in different bones and in different types of bone over the human lifetime. For example,

Pb accumulation is substantially higher in the compact portions of tibia than in other bones, increasing with age (Wittmers *et al.*, 1988; Aufderheide and Wittmers, 1992).

There are also reports that bone Pb can be used as a predictor of Pb's health effects and cumulative Pb exposure. In humans, studies found a relationship between bone Pb levels and prospective development of hypertension (Hu *et al.*, 1996; Cheng *et al.*, 2001), and child intelligence (IQ) (Wasserman *et al.*, 2003). In vultures, bone Pb concentrations were associated with an increased risk of bone fractures (Gangoso *et al.*, 2009). These findings indicate that bone Pb concentrations may provide a useful tool for assessing sub-lethal Pb exposure and possibly for determining whether Pb poisoning contributed to mortality in condors. Pb residency time is usually much longer in bones than in soft tissues. In humans, the mean life of Pb in soft tissues is 30 – 55 days and of over hundreds of days in bones (Rabinowitz, Wetherill and Kopple, 1976). Moreover, Pb uptake into bone depends on blood perfusion in bone and bone mineral formation (O'Flaherty, 1993).

Long bones, such as femur, are composed of a diaphysis with a rim of cortex and a hollow medullary cavity, flared cone-shaped metaphyses at the growth plates, and rounded epiphyses above the growth plates at either end of the bone (Clarke, 2008). The bone matrix comprising these different regions of long bones varies and can be categorized by the tissue architecture into compact and trabecular bone types. Compact bone comprises the cortical areas of long bone and is characterized by a relatively dense matrix (0.9 g/cm^3 in White Leghorn chickens), whereas trabecular bone comprises

epiphyseal and metaphyseal regions of long bone and has a mesh structure with relatively less dense composition (0.2 g/cm^3 in White Leghorn chickens) (Jendral *et al.*, 2008). In humans, diaphysis of femur is composed of ~95% compact bone and femur epiphyses are composed of ~50% trabecular and 50% compact bone (Clarke, 2008). It is known that compact and trabecular bones have different mineral turnover rates, for instance in White Leghorn hens bone mineral turnover rates are ~0.7% per month in compact bone and ~4% per month in trabecular bone, as determined by calcium (Ca) radio-isotope measurements for femur and tibiotarsus (Hurwitz, 1965). In avian species, some bones are pneumatized, meaning the medullary cavity does not contain marrow but rather membraneous sacs of air to reduce skeletal weight (Cubo and Casinos, 2000). In condors, humerus and femur are pneumatized, whereas tibiotarsus is not and contains marrow. The presence of marrow is associated with increased blood perfusion. Blood perfusion is known to affect Pb uptake into bone (O'Flaherty, 1993).

Findings from Chapter 2 showed that Pb concentrations vary greatly across different bones within the condor skeleton, and that those differences in bone Pb concentrations are not simply due to differences in bone mineral content. Bone regions that are expected to have faster bone mineral turnover rates, such as largely trabecular bone, and in particular bones that that contain marrow in condors, such as tibiotarsus, have higher Pb levels following Pb exposure. In contrast, bone regions that are expected to have slower bone mineral turnover rates, such as those comprised mostly of compact

bone (e.g., diaphysis of long bones), had relatively lower Pb levels following Pb exposure, compared to the largely trabecular bone regions. In this chapter, I investigated whether bone Pb concentrations in different bones could be used to inform Pb poisoning status at the time of death. Also, since, in humans, cumulative Pb exposure is known to be associated with health effects (Hu *et al.*, 1996; Cheng *et al.*, 2001), and largely compact bones are known to better reflect cumulative Pb exposure over the lifespan (Wittmers *et al.*, 1988; Aufderheide and Wittmers, 1992), I investigated whether monitoring variables that are often measured in free-flying birds – such as proportion of days in the wild observed feeding on proffered carcasses, periodic blood Pb monitoring, age, and other variables – are associated with bone Pb concentration.

In this chapter, I pursued three main objectives. First, determine if bone Pb levels in three different bones/bone regions can serve as predictive biomarker of Pb poisoning that can help diagnose whether birds died of Pb toxicosis or not. Second, determine whether bone Pb levels were associated with a decrease in bone mineral content in condors. And third, determine whether cumulative Pb exposure, as reflected in bone Pb levels, was associated with monitoring and life history variables that are measured in free-flying birds.

3.2 Methods

3.2.1 Sample selection and inclusion criteria

In order to explore how Pb levels differed across bones in condors with different Pb exposure histories, I selected 41 birds divided into four Pb exposure categories: 1) condors that were not free-flying for more than a few days (**Non-exposed**, n = 5, condors 32, 445, 502, 639, 699); 2) Pb poisoning was the diagnosed primary CoD (**Pb CoD**, n = 15, condors 112, 192, 238, 242, 245, 246, 272, 286, 306, 312, 318, 345, 458, 478, 664); 3) Birds with a diagnosed CoD other than Pb toxicosis (**Other CoD**, n = 9, condors 63, 125, 301, 307, 412, 511, 512, 615, 630); and 4) Birds with an unknown CoD (**UNK CoD**, n = 12, condors 102, 195, 265, 299, 356,408, 411, 499, 536, 553, 598, 668). Non-exposed birds either lived in captivity their entire life or were in the wild for less than a week before death and had a necropsy liver Pb concentration below the limit of detection, indicating that they were not Pb exposed in the brief time that they were in the wild. Birds in the latter three Pb exposure categories were categorized based on a board-certified pathologist necropsy report. The description of condors used is summarized in Table 3.1.

To guide the selection of bones to be used in this study, I relied on results from my previous dissertation chapter. In Chapter 2, I showed that epiphyses and diaphyses have different Pb concentrations depending on Pb exposure category. In particular, bone Pb concentrations were 2.8-fold higher in epiphyses than in diaphyses in condors that died of Pb poisoning, which could indicate that epiphyses are more sensitive to Pb

exposure events due to higher rates in Pb uptake than diaphyses. Epiphyses are composed of mostly trabecular bone and diaphysis of mostly compact bone (Clarke, 2008). Moreover, long bones are usually found on the extremities and as so could be sampled with minimum damage to the carcasses, which make long bones were good candidates for the study in this chapter. Since the diaphyses of humerus, femur and tibiotarsus presented similar Pb concentrations, based on findings in Chapter 2, I decided to use the tibiotarsus diaphysis. Also, since the proximal tibiotarsus epiphysis and the distal femur epiphysis showed the highest variability in Pb concentrations across condors (n = 12), likely reflecting differences in Pb exposure history proximal to death, I decided to use those two epiphyses in the present study. Therefore, the bones selected were proximal epiphysis of tibiotarsus, diaphysis of tibiotarsus and distal epiphysis of femur.

Inclusion criteria of cases in the present study were i) carcass was readily available (at UCSC or from SD Zoo or USFWS Ashland laboratory), ii) a CoD had been determined by pathologist, enabling classification into one of above defined CoD categories (Pb CoD, Other CoD, UNK CoD) or Non-exposed, iii) known Pb liver concentration from necropsy (except for Non-exposed birds), and iv) a selection of birds representing a large range of ages and time in the wild. Liver Pb concentration from the necropsy report were used for analysis, when the report indicated "below the detection limit," a value of 1 μg/g was attributed.

Specific condor monitoring variables demonstrated in a prior study to be associated with survival (Bakker *et al.*, 2016) were used. These variables include: Age (condor age at death); FreeFlyDays (number of days a condor was free flying); FeedonProffered (proportion of days a condor was in the wild and observed feeding on proffered carcasses with respect to the number of days it was free flying). Additional variables from blood Pb concentration monitoring data were collected and include: 18-month blood peak (highest blood Pb concentration in the 18 months prior to a condor's death); 18-month blood integral (the area under the curve when blood lead is plotted against time in the 18 months prior to a condor's death; Lifetime blood peak (highest blood Pb concentration over the lifetime of a condor); Lifetime blood integral (the area under the curve when blood lead is plotted against time over the lifetime of a condor). The complete list of variables and values for each condor is provided in the Appendix A Table S.2. Proffered feeding stations are used for monitoring and routine health checks purposes (USFWS, 2014). Monitoring of free-flying birds happens on a near-daily basis via visual observation of patagial wing tags or radio (VHF) and/or GPS transmitters signals (Walters *et al.*, 2010). Condors are also trapped approximately semi-annually for health checkups including blood lead assessment (Walters *et al.*, 2010).

Table 3.1 – Description of cases used in this study. Each case is uniquely identified by a studbook number, which is also routinely used for monitoring during the lifetime of the bird. Each condor is classified into a Pb exposure/cause of death category.

Studbook #	Category / Cause of Death[i]	Sex	Age (years)	Time in the wild[ii] (years)	Number of chelations[iii] (past year / lifetime)	Last known chelation[iv] (days prior to death)	Liver Pb at necropsy (µg/g)[v]	Most recent blood Pb (µg/dL)[vi]	Date of most recent blood Pb in relation to death[vi]
32	Non-exposed	Female	32	0	0/0	NA	NA	NA	NA
445	Non-exposed	Male	2.9	0	0/0	NA	NA	NA	NA
502	Non-exposed	Male	2.8	0	0/0	NA	NA	NA	NA
639	Non-exposed	Female	2.5	0.01	0/0	NA	< 1	NA	NA
699	Non-exposed	Male	0.7	0.69	0/0	NA	NA	NA	NA
63	Other CoD	Male	23.4	3.27	0/0	NA	< 1	3	103
125	Other CoD	Male	19.1	18.44	1/10	NA	NA	17	163
301	Other CoD	Male	4.1	2.6	1/1	NA	< 1	67	188
307	Other CoD	Female	4	2.55	1/3	NA	< 1	20	70
412	Other CoD	Male	5.7	5.88	0/3	NA	< 1	32	404
511	Other CoD	Male	1.6	0.12	NA	NA	NA	NA	NA
512	Other CoD	Male	3.7	1.63	0/0	NA	< 1	3	43
615	Other CoD	Male	14.6	3.04	2/2	NA	NA	20	19
630	Other CoD	Female	2.3	2.37	0/0	NA	< 1	25	71
112	Pb CoD	Female	17	14.27	2/11	4	20	190	5
192	Pb CoD	Female	17.3	16.67	0/9	NA	NA	11	448
238	Pb CoD	Male	7.1	6.11	1/2	4	< 1	80	4
242	Pb CoD	Male	14.3	12.64	0/3	NA	83	12	294
245	Pb CoD	Female	6.3	5.29	1/1	14	2	523	14
246	Pb CoD	Male	10.9	10.1	NA	NA	NA	NA	NA

272	Pb CoD	Male	13.8	12.87	NA	NA	NA	NA	NA
286	Pb CoD	Male	7	5.35	1/2	68	< 1	180	68
306	Pb CoD	Female	10.1	8.62	0/3	NA	52	3	357
312	Pb CoD	Female	9.9	8.45	0/3	NA	69	14	314
318	Pb CoD	Male	9.5	8.19	1/9	17	4.6	760	17
345	Pb CoD	Male	9	7.7	1/5	NA	42	13	132
458	Pb CoD	Female	2.8	1.5	0/0	NA	12	3	133
478	Pb CoD	Male	4	2.55	0/2	10	18	13	193
664	Pb CoD	Female	2.6	2.72	1/2	NA	29	27	76
102	Unknown	Male	6.5	5.63	1/2	46	NA	68	46
195	Unknown	Female	11.6	10.79	NA	NA	NA	NA	NA
265	Unknown	Male	13.4	11.53	NA	NA	NA	NA	NA
299	Unknown	Male	10.7	9.79	NA	NA	< 1	NA	NA
356	Unknown	Female	2.6	1.37	0/0	NA	NA	3	93
408	Unknown	Female	4.7	3.48	0/2	NA	NA	23	43
411	Unknown	Male	8.6	7.48	1/7	38	NA	100	38
499	Unknown	Female	2.6	2.71	1/1	49	< 1	38	49
536	Unknown	Female	4.3	2.89	0/0	NA	NA	23	62
553	Unknown - Suspected Pb	Male	2.7	0.83	NA	NA	NA	NA	NA
598	Unknown	Female	1.7	1.72	1/1	3	< 1	53	3
668	Unknown	Male	1.9	0.45	NA	NA	NA	NA	NA

[i] Birds were divided into four categories based on cause of death as determined by a board-certified pathologist on the necropsy report as well as birds that I defined as "Non-Exposed": 1) Non-exposed (birds that lived only in captivity or a few days in the wild); 2) Other CoD (birds that lived in the wild and were likely elevated exposed to Pb, but the cause of death was not Pb); 3) Pb CoD (birds whose cause of death was Pb). Condor 553 did not have a definitive cause of death, but the pathologist indicates that Pb toxicosis is suspected.

[ii] Time in the wild is the time that a condor was free flying, based on release date for birds that hatched in captivity, hatch date for birds that hatched in the wild, and date of death. The days that a condor was captured and kept in captivity for observation or treatment were subtracted from the total time in the wild.

[iii] Chelation is a chemical treatment that helps eliminate trace metals from blood. Chelation treatment was typically administered to condors when blood Pb levels higher than 35 μg/dL are measured during routine monitoring (USFWS, 2014). I report the number of times chelation treatment was applied in the year prior to a condor's death and over the lifetime of a condor. Each chelation regimen typically lasts for 1 to several weeks, depending on blood Pb level.

[iv] Start date for last known chelation treatment, reported in the necropsy.

[v] Liver Pb concentration (fresh weight) is routinely measured during necropsy to inform the pathologist about the Pb poisoning status at the time of death.

[vi] Blood Pb concentration is routinely measured biannually depending on a bird being captured by field teams. The latest available blood Pb concentration is reported here with respective date.

3.2.2 Sample collection, processing and analysis

Bone samples were collected from the selected bones, processed and analyzed in a high-efficiency particulate attenuated (HEPA) filtered air laboratory using established trace metal clean techniques, as described elsewhere (Smith et al. 1996; Finkelstein et al. 2003; Finkelstein et al. 2010). Briefly, bone samples were collected using a titanium corer fitted to a power drill, or Dremel saw fitted with a stainless steel blade. Bone samples (50 – 100 mg) were cleaned of adherent soft tissue with a stainless steel scalpel, rinsed with ultrapure grade water (Milli-Q system from Millipore, Inc), acetone and ultrapure 5% HNO3 acid. Subsamples were then dried in an oven for 3 days (65 °C) and weighed. Triplicates subsamples were used for each bone location. Bone samples were digested in 1 mL ultrapure concentrated HNO_3 in polytubes for 6 hours in a water bath (80 °C); after digestion, samples were cooled for ~30 minutes and 5 mL of ultrapure MQ water was added to dilute the samples.

Bone Pb concentrations were analyzed on quadrupole (Thermo XSeriesII) or magnetic sector inductively coupled plasma mass spectrometry (ICP-MS, Thermo Element XR magnetic sector), using ^{205}Tl as an internal standard. Between-run (i.e., long-term over several months) measurement precision was ~13% RSD (n = 8 runs), based on repeated measurements of three condor bone samples. National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 1486 (Bone Meal) and 1400 (Bone Ash) were used to validate accuracy of the bone Pb concentration measurements with recoveries of 80% – 99% (n = 24) for Bone Meal and

77% – 91% (n = 24) for Bone Ash. The analytical limit of detection for Pb measurements was 0.0047 ng/mL.

Calcium (Ca) and phosphorus (P) concentrations were measured by inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer Optima). National Institute of Standards and Technology (NIST) Standard Reference Materials (SRM) 1486 (Bone Meal) and 1400 (Bone Ash) were used to validate accuracy of measurements; the recoveries for bone Ca measurements were of 93% – 115% (n = 24) for Bone Meal and 92% – 109% (n = 24) for Bone Ash. Accuracy (recovery) of P was of 88% – 116% (n = 24) for Bone Meal and 89% – 111% (n = 24) for Bone Ash. The analytical limit of detection for Ca measurements was 0.014 μg/mL and for P it was 0.11 μg/mL.

3.2.3 Statistical analysis

Bone Pb concentration variation across the skeleton of condors: To determine whether there was an effect of Pb exposure/CoD category and bone region in bone Pb concentration, I performed a mixed model test using individual triplicates and normalized, log-transformed data, examining CoD category, bone region, and crossing of CoD category and bone region. Bone region, CoD category and the crossing of them were defined as fixed effects and Studbook # was defined as random effect (n = 36 birds, 324 samples). Samples from birds in the Non-exposed category were excluded from this analysis since they would bias the results towards a significant effect of category because the bone Pb levels in those birds were significantly lower than in

birds that were free-flying and exposed to above background levels of Pb. Tukey post-hoc tests were performed between each pair of bone regions (tibiotarsus epiphysis proximal X tibiotarsus diaphysis, tibiotarsus epiphysis proximal X femur epiphysis distal, and tibiotarsus diaphysis X femur epiphysis distal) within a Pb exposure category to determine whether Pb concentrations in those bone regions differed across that pair. Post-hoc Tukey tests were also performed between the same bone region across CoD categories to determine whether bone Pb concentration varied for that bone region between the categories.

To determine whether there was an effect of Pb exposure/CoD category and bone region in bone Ca concentration, I performed a mixed model test using individual triplicates and using CoD category, bone region, and the crossing of CoD category and bone region. Studbook # was set as a random effect (n = 41 birds, n = 369 samples). Post-hoc Tukey tests were performed between each pair of bone regions within a category to determine whether Ca concentration in those bone regions differed across that pair. Post-hoc Tukey tests were also performed between the same bone region across categories to determine whether bone Ca concentration varied for that bone region between the CoD categories. To check whether differences in CoD category could be explained by differences in age of the condor, I performed an ANOVA.

To explore whether the variation in Ca concentrations was reflective of variation in bone mineral content, I investigated Ca to P ratio in each sample and

performed a Spearman's correlation test between bone P and Ca concentration using individual triplicates and without splitting the data by category.

Bone Pb concentration as a biomarker of Pb exposure: To determine whether Pb concentrations in each bone region were correlated with each other and with Pb concentration in liver, I performed a Spearman's correlation (n = 41 birds for bones and n = 22 birds for liver), using the average of triplicates for each bone region. To determine whether bone Pb concentrations could be used to infer to which Pb exposure/CoD category a condor belongs, i.e., Pb CoD or Other CoD, I performed a discriminant analysis using only birds in the Pb CoD or Other CoD categories that had not been chelated in the 2 months prior to death (n = 18 birds). Chelation decreases blood Pb concentration and would affect the model generated by the discriminant analysis. The average and SD of the triplicates for each bone region were determined and are summarized in Table 3.2. To test the robustness of the discriminant classification, I also randomly selected one triplicate value from each bone region and condor and reran the analysis. The discriminant model, which used the average of triplicate bone lead values, was then applied to condors in the UNK CoD category and to condors that were chelated in the two months prior to death to determine to which Pb exposure/CoD category each condor should belong.

Bone Pb level and decrease in bone mineral density: To investigate whether there was a relationship between bone Pb concentration and bone mineral content, I performed a Spearman's regression between 1) bone Ca and bone Pb concentrations,

2) bone P and bone Pb concentrations, and 3) bone P concentrations and bone Pb:Ca ratios of both epiphyses and diaphysis of the bones, since those regions have different bone architecture. I also compared bone P and bone Ca concentration in epiphyses and diaphysis using a Student's t-test.

Cumulative Pb exposure and monitoring and life history variables: To explore which monitoring variables (Age, FreeFlyDays, FeedonProffered, 18 month blood peak, 18 month blood integral, Lifetime blood peak, Lifetime blood integral) are better correlated with bone Pb concentrations in each of the three bone regions used, Spearman's correlation tests were performed between each of the monitoring variables and Pb concentrations in each of the bone regions, using the average of triplicates for bone samples. To determine whether some of the monitoring variables are correlated with each other, I performed a Spearman correlation between monitoring variables. To statistically compare slopes between regressions of a monitoring variable and each bone Pb concentration in each bone region, an ANCOVA was used.

3.3 Results

3.3.1 Bone Pb concentration as a biomarker of Pb exposure

Bone Pb concentration variation in the skeleton of condors

Bone Pb concentrations varied across the three bone regions tested depending on Pb exposure history (Figure 3.1). In Non-exposed birds (Figure 3.1A), bone Pb concentrations averaged 0.562 μg/g and ranged between 0.082 and 1.60 μg/g. In Other CoD birds (Figure 3.1B), bone Pb concentrations averaged 12.8 μg/g and ranged

between 0.456 and 35.1 μg/g. A mixed model test using log transformed individual bone region triplicate (excluding the Non-Exposed category, n = 36 birds, n = 324 samples) showed that there was significant effect of Pb exposure/CoD category ($F(2, 33) = 5.71$, $P = 0.007$), of bone region ($F(2, 279) = 109.54$, $P < 0.0001$), and a significant effect of the interaction of category X bone region ($F(4, 279) = 7.87$, $P < 0.0001$). Therefore, bone Pb concentration varied by category, depending on bone region. Birds in the Non-exposed category were not included in this test, since Pb concentration in Non-exposed birds were over 30-fold lower than that of the other Pb exposure/CoD categories and would bias the model and statistical outcomes. No significant effect was observed between bone Pb concentration across bone regions for the Non-exposed birds ($F(2, 42) = 0.160$, $P = 0.852$). The average and standard deviation of triplicates of bone Pb concentration for each bird is summarized in Table 3.2.

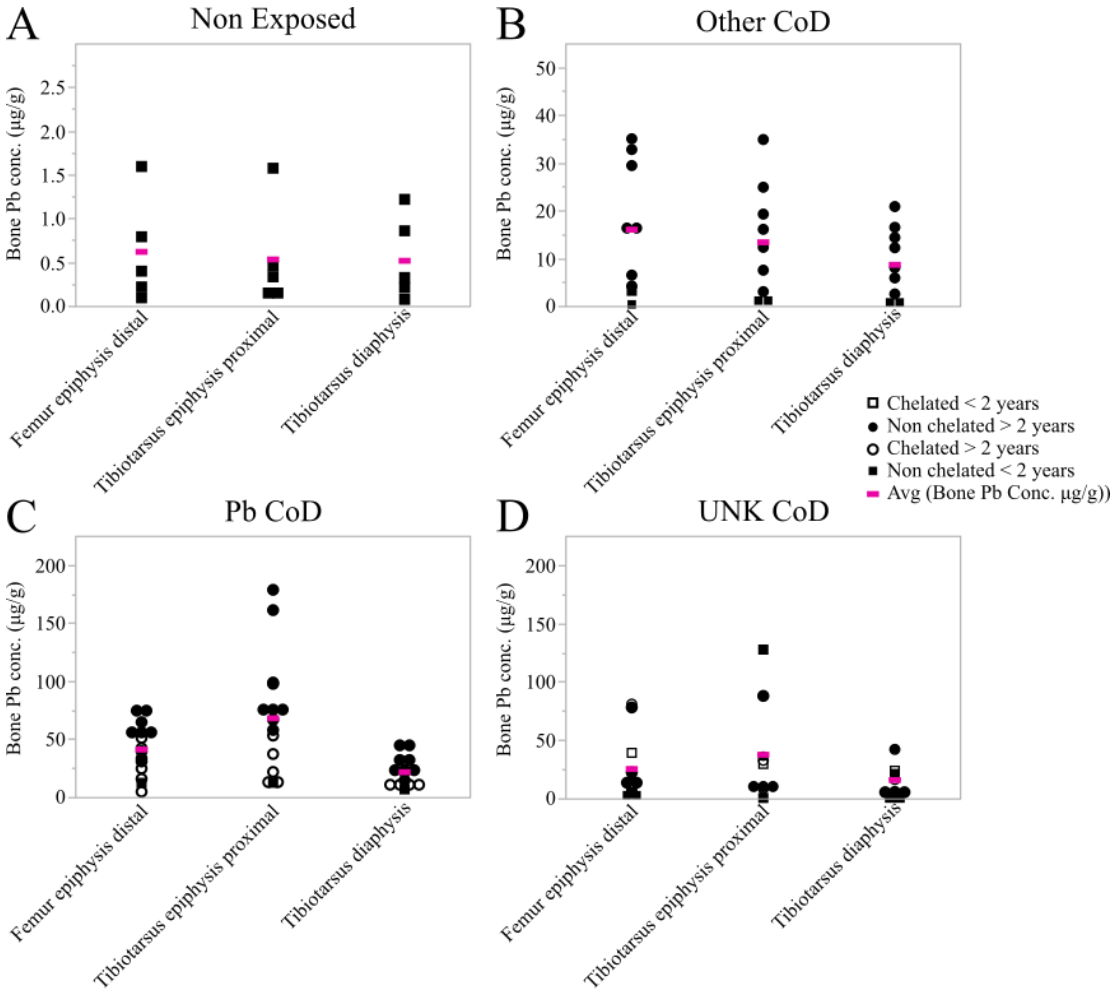


Figure 3.1 – Bone Pb concentration in three bone regions (femur epiphysis distal, tibiotarsus epiphysis proximal, tibiotarsus diaphysis) in four Pb exposure groups: (A) Non-exposed (n = 5 birds), (B) Other CoD (n = 9 birds), (C) Pb CoD (n = 15 birds), (D) UNK CoD (n = 12 birds). Each dot represents the average of triplicates for a single bird, and the pink dash represents the average across birds (total n = 41 birds). Round markers indicate birds that were in the wild for over two years and square markers indicate birds that were in the wild for less than two years. Filled markers indicate condors that were not chelated within 2 months of death and open markers indicate condors that were chelated within 2 months of death. ** indicate $P < 0.0001$, * indicate $P < 0.001$, and NS indicate no significance difference found.

In birds in the Other CoD category, there was considerable variability in bone Pb concentrations across birds, with an average of 8.75 µg/g with 81% RSD (range

0.502 to 21.0 μg/g) for tibiotarsus diaphysis, average of 16.1 μg/g with 84% RSD (range 0.456 to 35.1 μg/g) for femur epiphysis distal, and average of 13.5 μg/g with 86% RSD (range 0.635 to 35.0 μg/g) for tibiotarsus epiphysis proximal. A post-hoc Tukey test revealed that bone Pb concentrations were different for birds in the Other CoD category between femur epiphysis distal and tibiotarsus diaphysis ($P < 0.0001$, DF = 35), and between the tibiotarsus epiphysis proximal and tibiotarsus diaphysis ($P < 0.0001$, DF = 35), but not between femur epiphysis distal and tibiotarsus epiphysis proximal ($P = 0.10$, DF = 35).

In Pb CoD birds, bone Pb concentrations in femur epiphysis distal had an average of 41.4 μg/g with 54% RSD (range 5.12 to 77.4 μg/g), in tibiotarsus diaphysis had an average of 21.9 μg/g with 52% RSD (range 7.16 to 45.5 μg/g), and in tibiotarsus epiphysis proximal had an average of 68.3 μg/g with 75% RSD (range 10.6 to 178 μg/g). Tibiotarsus epiphysis proximal had the highest Pb concentrations across the bones tested in this group. Compared to the Other CoD category, there was less variability in bone Pb concentration across birds in this group: ~53% RSD for femur epiphysis distal and tibiotarsus diaphysis and ~75% for tibiotarsus epiphysis proximal. When performing a post-hoc Tukey test, significant differences were found between femur epiphysis distal and tibiotarsus diaphysis ($P < 0.0001$, DF = 35), tibiotarsus epiphysis proximal and femur epiphysis distal ($P < 0.0001$, DF = 35), and between tibiotarsus diaphysis and tibiotarsus epiphysis proximal ($P < 0.0001$, DF = 35).

In UNK CoD birds, bone Pb concentrations for femur epiphysis distal had an average of 25.6 μg/g with 112% RSD (range 1.34 to 81.2 μg/g), for tibiotarsus diaphysis had an average of 16.2 μg/g with 91% RSD (range 1.03 to 42.7 μg/g), and for tibiotarsus epiphysis proximal had an average of 37.9 μg/g with 108% RSD (range 0.852 to 128 μg/g). Significant differences were found when performing a post-hoc Tukey test between bone Pb concentrations in femur epiphysis distal and tibiotarsus diaphysis ($P < 0.0001$, $DF = 35$), tibiotarsus epiphysis proximal and femur epiphysis distal ($P = 0.0008$, $DF = 35$), and between tibiotarsus diaphysis and tibiotarsus epiphysis proximal ($P < 0.0001$, $DF = 35$).

Table 3.2 – Summary of bone Pb, Ca, and P concentrations by condor and by bone region used in this chapter. Each column contains average and SD of triplicates. Individual replicates are in Appendix A Table S.1.

Studbook #	Pb exposure / CoD Category	Bone Region	Bone Pb Conc. (avg ± SD µg/g)	Bone Ca Conc. (avg ± SD mg/g)	Bone P Conc. (avg ± SD mg/g)
32	Non-exposed	Femur epiphysis distal	1.60 ± 0.257	218 ± 8.08	95.6 ± 3.73
32	Non-exposed	Tibiotarsus diaphysis	1.23 ± 0.111	250 ± 11.5	109 ± 4.60
32	Non-exposed	Tibiotarsus epiphysis proximal	1.58 ± 0.095	170 ± 17.1	75.8 ± 6.77
445	Non-exposed	Femur epiphysis distal	0.100 ± 0.011	191 ± 6.49	83.2 ± 6.38
445	Non-exposed	Tibiotarsus diaphysis	0.082 ± 0.006	202 ± 20.7	90.9 ± 8.82
445	Non-exposed	Tibiotarsus epiphysis proximal	0.121 ± 0.006	194 ± 13.6	89.4 ± 5.63
502	Non-exposed	Femur epiphysis distal	0.404 ± 0.055	230 ± 8.45	102 ± 5.10
502	Non-exposed	Tibiotarsus diaphysis	0.227 ± 0.015	259 ± 2.93	114 ± 1.64
502	Non-exposed	Tibiotarsus epiphysis proximal	0.339 ± 0.017	158 ± 13.06	69.7 ± 3.88
639	Non-exposed	Femur epiphysis distal	0.798 ± 0.240	193 ± 15.7	89.9 ± 2.53
639	Non-exposed	Tibiotarsus diaphysis	0.866 ± 0.055	234 ± 5.07	102 ± 2.09
639	Non-exposed	Tibiotarsus epiphysis proximal	0.462 ± 0.135	153 ± 75.7	71.9 ± 12.1
699	Non-exposed	Femur epiphysis distal	0.225 ± 0.009	186 ± 4.9	85.9 ± 2.68
699	Non-exposed	Tibiotarsus diaphysis	0.215 ± 0.006	226 ± 7.28	105 ± 3.20
699	Non-exposed	Tibiotarsus epiphysis proximal	0.188 ± 0.034	138 ± 15.1	65.5 ± 8.04
63	Other CoD	Femur epiphysis distal	4.31 ± 0.642	231 ± 7.14	101 ± 3.03
63	Other CoD	Tibiotarsus diaphysis	2.64 ± 0.365	262 ± 5.16	115 ± 1.85
63	Other CoD	Tibiotarsus epiphysis proximal	3.12 ± 0.459	185 ± 18.2	84.5 ± 8.07
125	Other CoD	Femur epiphysis distal	32.9 ± 3.28	210 ± 4.02	93.6 ± 2.14
125	Other CoD	Tibiotarsus diaphysis	20.9 ± 2.05	237 ± 4.85	104 ± 3.04
125	Other CoD	Tibiotarsus epiphysis proximal	16.2 ± 2.17	125 ± 12.4	62.8 ± 9.45
301	Other CoD	Femur epiphysis distal	16.5 ± 0.281	182 ± 8.29	80.1 ± 4.33

301	Other CoD	Tibiotarsus diaphysis	6.36 ± 0.395	224 ± 34.6	98.8 ± 17.0
301	Other CoD	Tibiotarsus epiphysis proximal	12.4 ± 1.04	159 ± 14.14	71.9 ± 6.60
307	Other CoD	Femur epiphysis distal	29.5 ± 3.95	213 ± 10.8	95.3 ± 4.70
307	Other CoD	Tibiotarsus diaphysis	15.7 ± 1.68	253 ± 4.39	114 ± 2.25
307	Other CoD	Tibiotarsus epiphysis proximal	35.0 ± 0.930	188 ± 7.95	85 ± 3.05
412	Other CoD	Femur epiphysis distal	35.1 ± 3.61	193 ± 12.9	83.5 ± 5.59
412	Other CoD	Tibiotarsus diaphysis	12.9 ± 0.948	240 ± 1.36	105 ± 2.80
412	Other CoD	Tibiotarsus epiphysis proximal	19.4 ± 1.25	188 ± 8.40	81.9 ± 6.13
511	Other CoD	Femur epiphysis distal	0.457 ± 0.048	226 ± 7.14	102 ± 2.96
511	Other CoD	Tibiotarsus diaphysis	0.502 ± 0.076	233 ± 18.0	105 ± 7.25
511	Other CoD	Tibiotarsus epiphysis proximal	0.635 ± 0.112	205 ± 4.12	93.8 ± 2.18
512	Other CoD	Femur epiphysis distal	3.30 ± 0.525	207 ± 5.83	93.8 ± 2.26
512	Other CoD	Tibiotarsus diaphysis	1.25 ± 0.124	255 ± 5.10	114 ± 2.34
512	Other CoD	Tibiotarsus epiphysis proximal	1.86 ± 0.166	154 ± 12.9	71.4 ± 5.99
615	Other CoD	Femur epiphysis distal	16.3 ± 0.778	192 ± 18.7	88.5 ± 7.30
615	Other CoD	Tibiotarsus diaphysis	12.3 ± 1.53	228 ± 24.6	105 ± 8.64
615	Other CoD	Tibiotarsus epiphysis proximal	25.0 ± 0.848	155 ± 10.89	71.6 ± 4.91
630	Other CoD	Femur epiphysis distal	6.58 ± 0.893	199 ± 7.62	90.4 ± 3.07
630	Other CoD	Tibiotarsus diaphysis	6.00 ± 0.479	224 ± 13.11	102 ± 6.76
630	Other CoD	Tibiotarsus epiphysis proximal	7.62 ± 0.142	181 ± 20.9	84.4 ± 9.68
112	Pb CoD	Femur epiphysis distal	51.4 ± 4.24	211 ± 12.7	94.1 ± 5.34
112	Pb CoD	Tibiotarsus diaphysis	27.4 ± 1.67	236 ± 18.6	107 ± 8.11
112	Pb CoD	Tibiotarsus epiphysis proximal	53.5 ± 5.65	259 ± 106.1	118 ± 48.5
192	Pb CoD	Femur epiphysis distal	57.1 ± 12.1	212 ± 4.94	95.0 ± 2.57
192	Pb CoD	Tibiotarsus diaphysis	44.6 ± 8.38	234 ± 5.67	106 ± 1.98
192	Pb CoD	Tibiotarsus epiphysis proximal	99.1 ± 9.94	197. ± 10.7	90.2 ± 5.16
238	Pb CoD	Femur epiphysis distal	5.11 ± 0.729	229 ± 90.0	99.7 ± 35.9

238	Pb CoD	Tibiotarsus diaphysis	13.4 ± 3.36	199 ± 13.8	89.9 ± 6.63
238	Pb CoD	Tibiotarsus epiphysis proximal	10.6 ± 3.79	134 ± 40.5	58.7 ± 12.1
242	Pb CoD	Femur epiphysis distal	31.1 ± 1.28	277 ± 37.4	122 ± 16.5
242	Pb CoD	Tibiotarsus diaphysis	21.5 ± 3.77	257 ± 10.8	114 ± 4.88
242	Pb CoD	Tibiotarsus epiphysis proximal	75.9 ± 4.42	193 ± 2.63	87.2 ± 0.448
245	Pb CoD	Femur epiphysis distal	16.3 ± 0.248	213 ± 4.78	95.3 ± 1.91
245	Pb CoD	Tibiotarsus diaphysis	10.1 ± 2.19	220 ± 26.5	101 ± 9.76
245	Pb CoD	Tibiotarsus epiphysis proximal	19.3 ± 1.27	168 ± 14.3	77.5 ± 6.04
246	Pb CoD	Femur epiphysis distal	58.2 ± 8.92	211 ± 1.00	95.7 ± 0.756
246	Pb CoD	Tibiotarsus diaphysis	29.6 ± 1.72	246 ± 0.87	111 ± 0.488
246	Pb CoD	Tibiotarsus epiphysis proximal	63.3 ± 5.16	188 ± 5.80	87.2 ± 2.87
272	Pb CoD	Femur epiphysis distal	36.0 ± 1.42	215 ± 1.49	97.2 ± 0.743
272	Pb CoD	Tibiotarsus diaphysis	21.4 ± 2.13	253 ± 5.29	114 ± 1.06
272	Pb CoD	Tibiotarsus epiphysis proximal	70.3 ± 19.9	152 ± 36.03	71.9 ± 14.9
286	Pb CoD	Femur epiphysis distal	22.0 ± 7.39	204 ± 8.31	91.2 ± 3.54
286	Pb CoD	Tibiotarsus diaphysis	9.03 ± 0.348	262 ± 7.87	117 ± 3.58
286	Pb CoD	Tibiotarsus epiphysis proximal	16.1 ± 2.52	196 ± 7.00	90.4 ± 3.24
306	Pb CoD	Femur epiphysis distal	53.4 ± 16.5	213 ± 6.12	91.1 ± 2.52
306	Pb CoD	Tibiotarsus diaphysis	14.9 ± 1.75	234 ± 16.2	102 ± 7.83
306	Pb CoD	Tibiotarsus epiphysis proximal	161 ± 38.4	175 ± 5.16	77.7 ± 1.37
312	Pb CoD	Femur epiphysis distal	77.4 ± 22.6	215 ± 7.64	95.9 ± 2.64
312	Pb CoD	Tibiotarsus diaphysis	45.5 ± 6.32	259 ± 10.1	116 ± 5.99
312	Pb CoD	Tibiotarsus epiphysis proximal	179 ± 37.6	173 ± 5.77	81.2 ± 0.800
318	Pb CoD	Femur epiphysis distal	41.3 ± 5.36	209 ± 16.7	90.2 ± 4.16
318	Pb CoD	Tibiotarsus diaphysis	22.0 ± 1.36	244 ± 9.37	108 ± 4.97
318	Pb CoD	Tibiotarsus epiphysis proximal	98.0 ± 23.9	160 ± 12.9	74.9 ± 7.14
345	Pb CoD	Femur epiphysis distal	72.4 ± 19.9	195 ± 12.08	86.1 ± 5.60

345	Pb CoD	Tibiotarsus diaphysis	26.9 ± 1.81	230 ± 3.58	101 ± 1.86
345	Pb CoD	Tibiotarsus epiphysis proximal	58.3 ± 4.51	186 ± 36.3	85.3 ± 16.5
458	Pb CoD	Femur epiphysis distal	12.7 ± 1.59	230 ± 4.84	102 ± 3.56
458	Pb CoD	Tibiotarsus diaphysis	7.16 ± 2.13	237 ± 16.7	107 ± 7.69
458	Pb CoD	Tibiotarsus epiphysis proximal	12.9 ± 2.49	188 ± 18.1	84.6 ± 9.63
478	Pb CoD	Femur epiphysis distal	25.4 ± 2.80	193 ± 43.5	90.2 ± 19.9
478	Pb CoD	Tibiotarsus diaphysis	12.1 ± 1.42	224 ± 11.1	99.3 ± 2.80
478	Pb CoD	Tibiotarsus epiphysis proximal	37.6 ± 12.7	183 ± 8.14	85.6 ± 3.48
664	Pb CoD	Femur epiphysis distal	61.8 ± 29.2	182 ± 12.9	83.1 ± 6.88
664	Pb CoD	Tibiotarsus diaphysis	23.5 ± 0.693	224 ± 13.0	100 ± 3.69
664	Pb CoD	Tibiotarsus epiphysis proximal	69.7 ± 5.13	164 ± 23.2	76.6 ± 11.5
102	UNK CoD	Tibiotarsus diaphysis	16.7 ± 2.30	252 ± 6.65	114 ± 3.59
102	UNK CoD	Tibiotarsus epiphysis proximal	33.1 ± 17.1	111 ± 50.5	51.6 ± 23.4
195	UNK CoD	Femur epiphysis distal	10.7 ± 0.701	210 ± 16.2	98.2 ± 6.88
195	UNK CoD	Tibiotarsus diaphysis	6.39 ± 2.21	218 ± 78.6	97.7 ± 34.7
195	UNK CoD	Tibiotarsus epiphysis proximal	12.2 ± 2.04	160 ± 32.0	75.3 ± 14.6
265	UNK CoD	Femur epiphysis distal	19.2 ± 9.34	180 ± 76.9	80.8 ± 34.4
265	UNK CoD	Tibiotarsus diaphysis	20.4 ± 1.20	273 ± 0.89	124 ± 1.23
265	UNK CoD	Tibiotarsus epiphysis proximal	36.7 ± 6.01	173 ± 12.2	81.0 ± 5.70
299	UNK CoD	Femur epiphysis distal	78.4 ± 21.5	224 ± 16.8	100 ± 7.41
299	UNK CoD	Tibiotarsus diaphysis	42.7 ± 6.69	251 ± 7.36	112 ± 3.40
299	UNK CoD	Tibiotarsus epiphysis proximal	87.9 ± 10.8	174 ± 22.6	80.4 ± 9.95
356	UNK CoD	Femur epiphysis distal	1.34 ± 0.118	208 ± 12.2	93.6 ± 5.30
356	UNK CoD	Tibiotarsus diaphysis	1.03 ± 0.407	196 ± 73.7	89.3 ± 33.7
356	UNK CoD	Tibiotarsus epiphysis proximal	0.853 ± 0.121	135 ± 19.5	63.6 ± 8.62
408	UNK CoD	Femur epiphysis distal	15.4 ± 1.82	198 ± 24.6	90.4 ± 9.16
408	UNK CoD	Tibiotarsus diaphysis	6.87 ± 1.64	247 ± 0.334	112 ± 0.263

408	UNK CoD	Tibiotarsus epiphysis proximal	10.6 ± 0.608	167 ± 6.84	75.1 ± 1.53
411	UNK CoD	Femur epiphysis distal	81.2 ± 16.03	214 ± 15.3	95.2 ± 6.11
411	UNK CoD	Tibiotarsus diaphysis	42.5 ± 3.24	262 ± 9.63	116 ± 4.31
411	UNK CoD	Tibiotarsus epiphysis proximal	88.3 ± 13.6	136 ± 18.06	61.4 ± 8.61
499	UNK CoD	Femur epiphysis distal	8.64 ± 2.10	223 ± 9.18	102 ± 4.47
499	UNK CoD	Tibiotarsus diaphysis	6.02 ± 0.288	261 ± 2.86	119 ± 1.16
499	UNK CoD	Tibiotarsus epiphysis proximal	8.83 ± 1.16	148 ± 1.70	70.9 ± 1.04
536	UNK CoD	Femur epiphysis distal	5.17 ± 0.626	226 ± 10.2	103 ± 4.74
536	UNK CoD	Tibiotarsus diaphysis	4.00 ± 0.602	258 ± 11.6	118 ± 5.54
536	UNK CoD	Tibiotarsus epiphysis proximal	9.04 ± 0.471	176 ± 11.2	80.9 ± 5.43
553	UNK CoD	Femur epiphysis distal	17.2 ± 2.39	214 ± 6.40	96.6 ± 2.85
553	UNK CoD	Tibiotarsus diaphysis	22.1 ± 1.07	211 ± 9.53	96.1 ± 1.18
553	UNK CoD	Tibiotarsus epiphysis proximal	128 ± 8.01	169 ± 15.7	78.7 ± 9.45
598	UNK CoD	Femur epiphysis distal	39.5 ± 3.56	223 ± 8.48	101 ± 4.28
598	UNK CoD	Tibiotarsus diaphysis	24.2 ± 0.879	254 ± 5.41	115 ± 2.39
598	UNK CoD	Tibiotarsus epiphysis proximal	29.9 ± 1.43	166 ± 11.09	77.3 ± 4.69
668	UNK CoD	Femur epiphysis distal	5.16 ± 3.14	351 ± 17.0	166 ± 7.74
668	UNK CoD	Tibiotarsus diaphysis	1.07 ± 0.130	253 ± 12.8	117 ± 6.21
668	UNK CoD	Tibiotarsus epiphysis proximal	9.07 ± 3.08	179 ± 19.8	86.9 ± 9.92

Bone Ca concentration in the skeleton of condors

Bone Ca concentrations had a wide range of values across the three bones tested (Figure 3.2A). For femur epiphysis distal had an average Ca concentration of 214 mg/g with 9.7% RSD and ranged between 180 and 277 mg/g, for tibiotarsus diaphysis had an average of 240 mg/g bone Ca concentration (8.3% RSD), ranging between 196 and 273 mg/g and tibiotarsus epiphysis proximal had an average of 170 mg/g (14.6% RSD), ranging between 101 and 205 mg/g. A mixed model using individual triplicates and that had as fixed effects category, bone region and the crossing of category X bone region and as random effect Studbook # (n = 41 birds, n = 369 samples) showed that there is a significant effect of bone region ($F(2, 307) = 398.16$, $P < 0.0001$), but not of category ($F(3, 37) = 0.78$, $P = 0.512$), which was expected since bone Ca is known to vary by bone and bone region and was not expected to vary by Pb exposure/CoD category. However, a significant interaction between category and bone region was found ($F(6, 307) = 5.15$, $P < 0.0001$). To check whether this effect could be due to differences in age of the birds within each of the four Pb exposure/CoD categories, I performed an ANOVA that showed that there was no significant difference in age of birds between the four Pb exposure/CoD categories ($F(3, 37) = 0.622$, $P = 0.606$).

Since bone Ca concentrations varied across bone regions for the samples tested, I explored whether the variation in Ca concentrations was reflective of variation in bone mineral content by investigating Ca:P ratio in each sample. As expected, Ca and P concentrations (Figure 3.2B) were highly correlated (Spearman's rho = 0.990, $P < 0.0001$, DF = 368). Moreover, the Ca:P atomic ratio observed from the slope of the

linear regression for all samples was 0.56 mol P/ mol Ca (Figure 3.2B), which was within the expected range ~0.53 – 0.64 mol P/mol Ca (Singh et al. 2007).

I also looked into the correlation between bone Pb and Pb:Ca ratio to determine whether the variation in bone Pb concentrations between bone regions was influenced by the variation in bone mineral content (Figure 3.2C). I found that bone Pb concentrations and the bone Pb:Ca ratio were highly correlated (Spearman's rho = 0.990, $P < 0.0001$, DF = 368). Since there was high correlation between bone Pb and bone Pb:Ca ratio, either variable can be used to reflect bone Pb content.

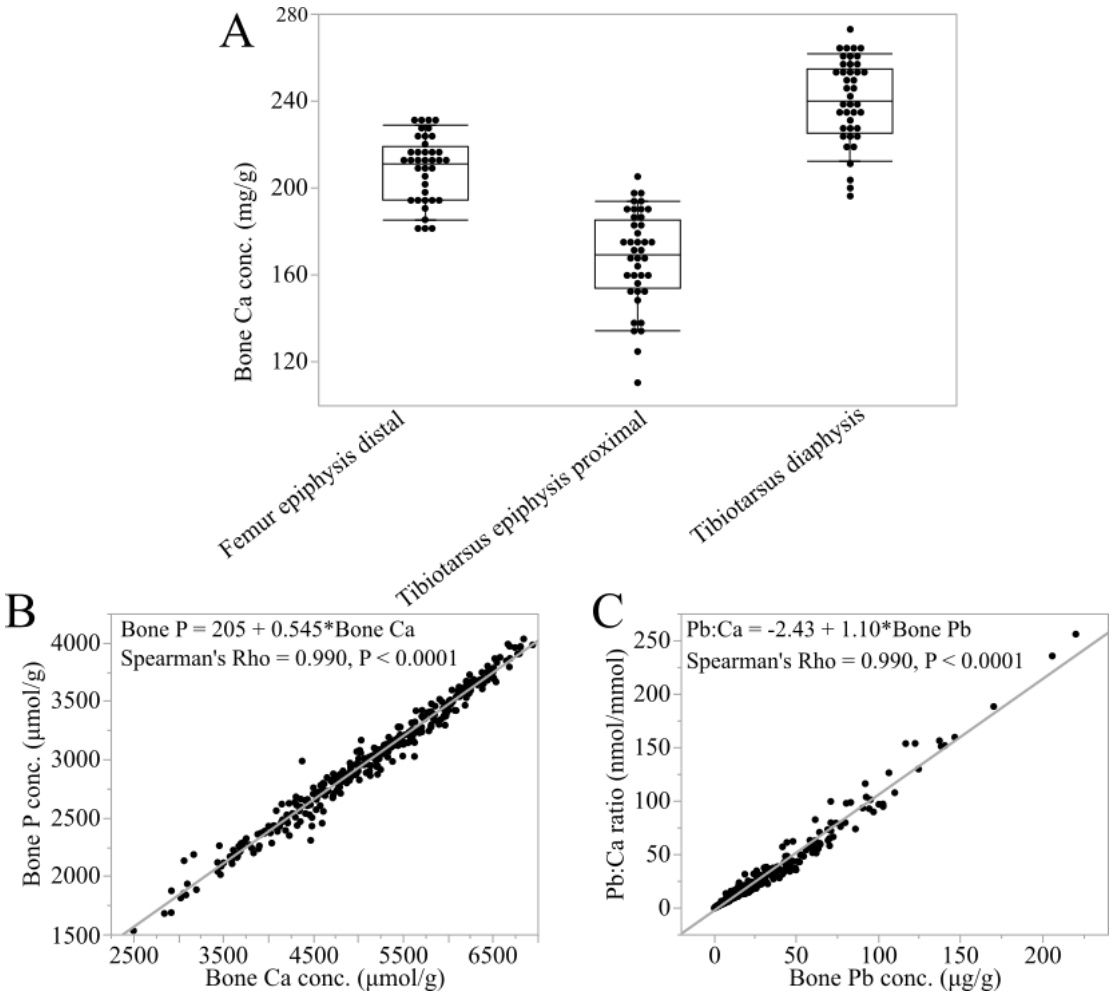


Figure 3.2 – (A) Bone Ca concentrations in three bone regions (femur epiphysis distal, tibiotarsus diaphysis, tibiotarsus epiphysis proximal), each dot represents the average of triplicates for a condor, and the Tukey box represents median (mid-box horizontal line), first (bottom end of box) and third (top end of box) quartiles, and the 1.5-times interquartile range (bottom and top whiskers) for the distribution. (B) Bone P concentration vs bone Ca concentration, each dot represents a single replicate measurement (n = 366 bones, 41 birds), the line represents the linear regression for the data, and the Spearman's correlation is indicated in the figure. (C) Bone Pb:Ca ratio vs bone Pb concentration, each dot represents a single replicate measurement (n = 366 bone samples), the line represents the linear regression for the data, and the Spearman's correlation is indicated in the figure.

3.3.2 Bone Pb concentration as a biomarker of Pb exposure

I found that Pb concentrations in the three bone regions were well correlated (R = 0.980, P < 0.0001, DF = 40, for all bone pairs) (Figure 3.3 and Table 3.3). Pb concentrations in the three bone regions were also correlated with liver Pb concentration; the tibiotarsus epiphysis proximal had the highest correlation (Spearman's rho = 0.713, P = 0.0002 DF = 21), followed by femur epiphysis distal (R = 0.576, P = 0.0005, DF = 21), and tibiotarsus diaphysis (R = 0.557, P = 0.007, DF = 21) (Figure 3.3 and Table 3.3).

Table 3.3 – Spearman's correlation between bones/bone regions and liver used in this study, using average of triplicate measurements within each bone and bone region. First line in each cell is the Spearman's rho, while the P value statistical significance and n number of birds (n) are shown in parentheses.

	Femur Epi Dist	**Tibiotarsus Diaphysis**	**Tibio Epi Prox**
Tibiotarsus Diaphysis	0.945 (<0.0001, n = 41)		
Tibio Epi Prox	0.926 (<0.0001, n = 41)	0.936 (<0.0001, n = 41)	
Liver	0.576 (0.005, n = 22)	0.557 (0.007, n = 22)	0.713 (0.0002, n = 22)

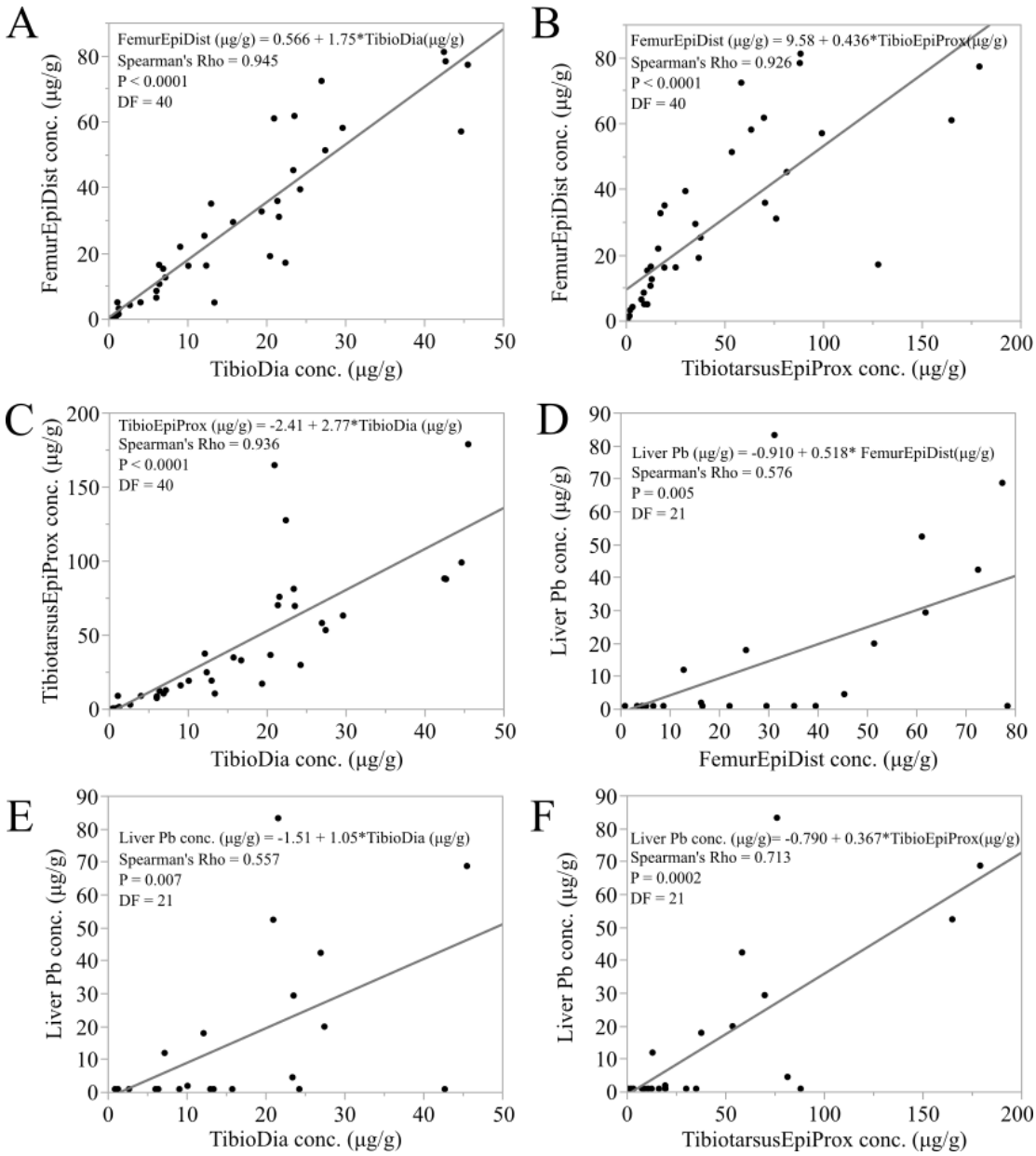


Figure 3.3 – Regression plots between: (A) bone Pb concentration in femur epiphysis distal and tibiotarsus diaphysis, (B) bone Pb concentration in femur epiphysis distal and tibiotarsus epiphysis proximal, (C) bone Pb concentration in tibiotarsus diaphysis and tibiotarsus epiphysis proximal; and between liver Pb concentration and: (D) bone Pb concentration in femur epiphysis distal, (E) bone Pb concentration in tibiotarsus diaphysis, (F) bone Pb concentration in tibiotarsus epiphysis proximal. Each point represents the average of replicates for bone Pb for each bird (n = 41 birds), the RSD

between replicates for each bird ranged between 2% and 61%, and single liver Pb concentration measurement (n = 22 birds).

To determine whether bone Pb concentration in each bone region was equally predictive of Pb exposure status at the time of death, I performed discriminant analyses using Pb concentrations in each bone individually (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis) to classify birds between the Other CoD and Pb CoD categories, using the average of triplicates. To prevent the interference of chelation in the results, birds that were chelated within two months of death were not included in this analysis (Pb, n = 6). Qualitatively the discriminant analysis with tibiotarsus epiphysis proximal had the highest rate of correctly classified birds, ~94%, misclassifying only one bird out 18 (Figure 3.4). The second highest rate of correctly classified birds was obtained by tibiotarsus diaphysis, ~89%, misclassifying two birds out 18 (Figure 3.4). Finally, femur epiphysis distal had the lowest rate of correctly classified birds, ~83%, misclassifying three birds out 18 (Figure 3.4); it is not clear if these differences in classification rate are statistically different. Performing a discriminant analysis considering more bone regions, in addition to tibiotarsus epiphysis proximal, did not improve the correctly classified rate: 94% of correctly classified birds, misclassifying one bird out 18 (Figure 3.4). Condor 458 was the only bird consistently misclassified by all the analyses.

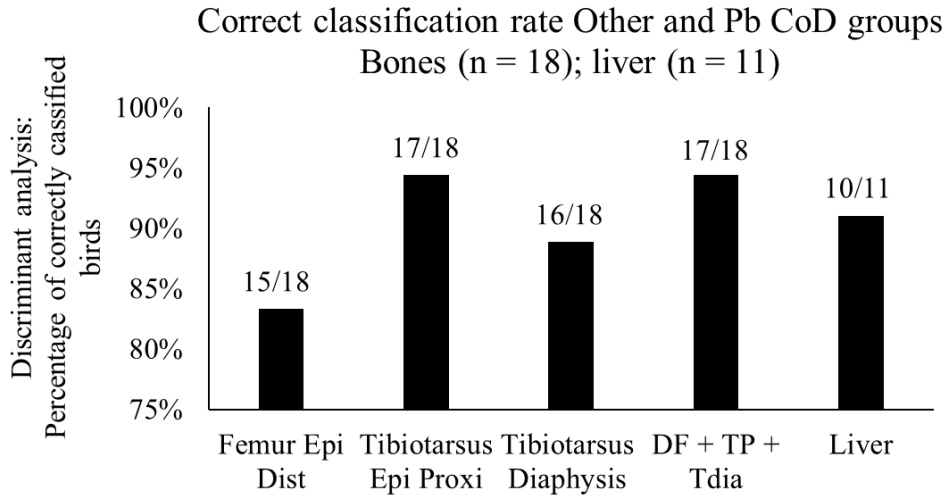


Figure 3.4 – Percentage of correctly classified birds using discriminant analysis using the average of replicates from each bone region and a single discriminant analysis that included all three bone regions: femur epiphysis distal (DF), tibiotarsus epiphysis proximal (TP), tibiotarsus diaphysis (Tdia). Birds that were chelated in the two months prior to death (Table 3.1) were not included in the analysis. For this plot where n = 18 birds, one bird is equivalent to 5.6% of classification.

In order to assess the confidence of category classification by the discriminant analyses, I evaluated the statistical probability of classification yielded in the analyses. For this, the probability that a condor is classified as Pb CoD is given by the discriminant analysis as a function of Pb concentration in the bone regions used the formula: $Prob_{Pb} = 1/(1 + e^{1/2(A-B.Pb_{bone})})$, where $Prob_{Pb}$ is the probability that a condor is classified as belonging to Pb CoD, Pb_{bone} is the bone Pb concentration in the bone region considered, and A and B are constants determined by the discriminant analysis using the condors with known CoD. For femur epiphysis distal the probability that a condor was in the Pb CoD group can be calculated as $Prob_{Pb} = 1/(1 + e^{1/2(7.81-0.227Pb_{femurDist})})$. For tibiotarsus epiphysis proximal the probability that a

condor was in the Pb CoD group can be calculated as $Prob_{Pb} = 1/(1 + e^{1/2(5.01-0.099Pb_{tibioProx})})$. For tibiotarsus diaphysis the probability that a condor was in the Pb CoD group can be calculated as $Prob_{Pb} = 1/(1 + e^{1/2(7.10-0.392Pb_{diaphysis})})$. When using the three bone regions, the probability that a condor was Pb CoD can be calculated as $Prob_{Pb} = 1/(1 + e^{1/2(8.21-0.168Pb_{femurDist}-0.090Pb_{diaphysis}+0.019Pb_{tibioProx})})$. For liver the probability that a condor was in the Pb CoD group can be calculated as $Prob_{Pb} = 1/(1 + e^{1/2(8.05-0.383Pb_{liver})})$. The probability that a condor is in the Other CoD category can be found as $Prob_{Other} = 1 - Prob_{Pb}$ in all the cases. The probabilities for the condors in the Pb and Other CoD categories are summarized in Table 3.4. Adding extra bones seemed to slightly improve the confidence of the classification (Table 3.4).

Even though the discriminant analysis using Pb concentration in more than one bone region did not improve the classification accuracy, the probability at which the condors were classified properly improved. In fact there was a significant difference (T = 2.45, P = 0.011, DF = 23) between the classification probability between the discriminant analysis using only bone Pb concentration in tibiotarsus epiphysis proximal and when using all three bone regions, indicating that in cases where the prediction probability is low (close to 50%), adding more bone regions to the analysis may help increase the confidence of the prediction outcome.

Table 3.4 – Probability of classification for each condor and each of the discriminant analysis performed using the average of replicates. Birds that were chelated in the two months prior to death (Table 3.1) were not included in the analysis (n = 18 birds). Stars indicate condors that were misclassified in each test. DF: Femur epiphysis distal, TP: Tibiotarsus epiphysis proximal, Tdia: Tibiotarsus diaphysis.

Studbook #	Category	Femur Epi Dist	Tibiotarsus Epi Proxi	Tibiotarsus Diaphysis	DF + TP + Tdia	Liver
63	Other CoD	96.8%	91.3%	95.4%	97.5%	97.9%
125	Other CoD	55.0%	84.6%	63.6%*	68.6%	NA
301	Other CoD	88.4%	86.9%	90.9%	92.5%	97.9%
307	Other CoD	63.6%	68.5%	61.4%	68.4%	97.9%
412	Other CoD	52.0%*	82.5%	73.3%	71.4%	97.9%
511	Other CoD	97.9%	92.2%	96.9%	98.3%	NA
512	Other CoD	97.2%	91.8%	96.5%	97.8%	97.9%
615	Other CoD	88.7%	78.1%	75.6%	87.7%	NA
630	Other CoD	95.9%	89.4%	91.5%	96.3%	97.9%
192	Pb CoD	92.9%	91.7%	99.5%	97.6%	NA
242	Pb CoD	59.3%*	77.7%	66.3%	63.1%	NA
246	Pb CoD	93.6%	65.1%	90.5%	90.6%	NA
272	Pb CoD	54.3%	66.5%	65.4%	63.6%	NA
306	Pb CoD	98.0%	99.7%	85.0%	99.5%	99.8%
312	Pb CoD	99.2%	99.8%	99.5%	99.9%	99.9%
345	Pb CoD	98.7%	59.3%	85.0%	94.8%	98.4%
458	Pb CoD	92.2%*	86.6%*	89.5%*	93.7%*	84.9%
664	Pb CoD	95.7%	72.0%	74.2%	91.4%	83.4%

To determine whether the approach is robust regardless of which triplicate subsample was used from a specific bone region, I repeated the discriminant analysis, but selecting a random replicate value to represent the bone Pb concentration. In practice, a single sample could be extracted and used for analysis, which is mimicked by the random replicate usage. The discriminant analysis using the randomly selected replicate values yielded similar accuracy as the discriminant analysis using the average of replicates: tibiotarsus epiphysis proximal ~94% correctly classified birds (17/18), tibiotarsus diaphysis, ~89% (16/18), and femur epiphysis distal ~78% (14/18). As

before, condor 458 was the only bird consistently misclassified by all the analysis. A single difference was found for femur epiphysis distal, where four out of 18 birds were misclassified, as opposed to three in the analyses using the average of the triplicates.

I also performed this analysis using liver Pb concentration, the current gold standard for Pb poisoning diagnoses. To prevent the interference of chelation in the results, birds that were chelated within two months prior to death were not included in this analysis (Pb, n = 6). The discriminant analysis using liver Pb concentration was able to correctly classify 10 out of 11 birds, with only condor 458 being misclassified (Figure 3.4).

Based on the results obtained from the discriminant analysis using birds whose cause of death is known, and since tibiotarsus epiphysis proximal and tibiotarsus diaphysis were the bone regions yielding slightly higher rates of correctly classified birds (17 and 16 out of 18 birds respectively), I applied two different discriminant analysis models, one generated using Pb concentrations from tibiotarsus epiphysis proximal and the other using Pb concentrations from tibiotarsus diaphysis to determine how each condor from the UNK CoD category would be classified according to the model, Pb or Other CoD. Applying the discriminant analysis model generated with the data from tibiotarsus epiphysis proximal, I found that three out of 12 condors in the UNK CoD category (299, 411, 553) were predicted as Pb CoD, they all had Pb concentrations in tibiotarsus epiphysis proximal >80 μg/g (Figure 3.5). Nine out of 12 condors in the UNK CoD category (356, 499, 536, 668, 408, 195, 598, 102, 265) were predicted as Other CoD, and they all had Pb concentrations in tibiotarsus epiphysis

proximal <40 µg/g (Figure 3.5). The probability that each condor is in the group predicted is given in Table 3.5 and was calculated using the formulas above; probability of classification ranged from 63.2% to 97.9% when using Pb concentration in tibiotarsus epiphysis proximal and between 54.7% to 99.1% when using Pb concentration in tibiotarsus diaphysis (Table 3.5). When considering bone Pb concentration in the tibiotarsus diaphysis, five out of 12 birds were classified as Pb CoD (265, 299, 411, 553, 598), whereas seven out of 12 birds were classified as Other CoD (356, 499, 536, 668, 408, 195, 102) (Figure 3.5).

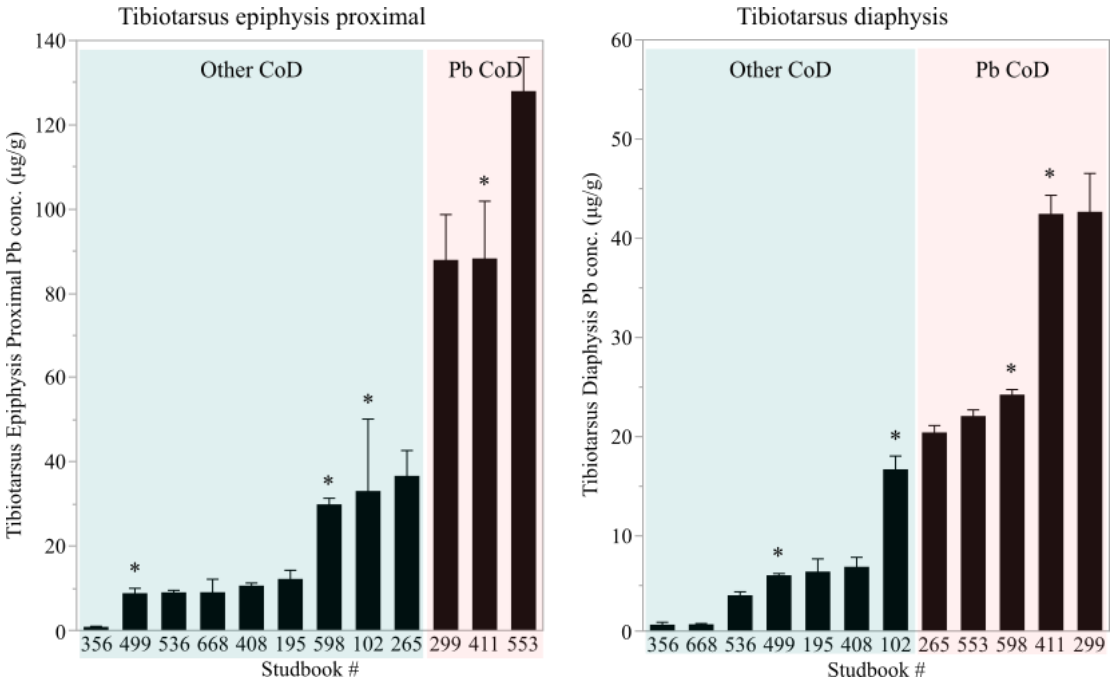


Figure 3.5 – Classification of UNK CoD birds (n = 12 birds) according to discriminant analysis using Pb concentration in tibiotarsus epiphysis proximal (left) and tibiotarsus diaphysis (right). The bars are the average of the triplicates and the error bar is the standard deviation. The shaded areas indicate how birds were classified and asterisks mark birds that were chelated in the two months prior to death.

Table 3.5 – Classification of UNK CoD condors according to discriminant analysis using Pb concentration in tibiotarsus epiphysis proximal (n = 12 birds) and tibiotarsus diaphysis (n = 12 birds) as well as model confidence.

Studbook #	Last chelation[i] (days)	Tibiotarsus epiphysis proximal		Tibiotarsus diaphysis	
		Predicted[ii]	Confidence[iii]	Predicted	Confidence
102	46	Other	63.2%	Other	54.7%
195	NR	Other	87.0%	Other	89.6%
265	NR	Other	66.6%	Pb	62.8%
299	NR	Pb	86.3%	Pb	99.1%
356	NR	Other	92.2%	Other	96.0%
408	NR	Other	87.9%	Other	89.0%
411	38	Pb	86.5%	Pb	99.1%
499	49	Other	88.8%	Other	90.2%
536	NR	Other	88.7%	Other	93.1%
553	NR	Pb	97.9%	Pb	71.0%
598	3	Other	73.6%	Pb	77.7%
668	NR	Other	88.7%	Other	96.0%

[i] Number of days since the start of the last known chelation treatment, reported in the necropsy report. NR indicate condors that were not chelated within the two months prior to its death.

[ii] Category predicted by the discriminant analysis model created using bone Pb concentration from the Other CoD and Pb CoD condors.

[iii] Estimated probability of the observation's actual classification, i.e., the probability that the condor belongs to the predicted category, for the UNK CoD condor's classification.

3.3.3 Pb effect on bone mineral content

Bone P concentration was on average 3470 μmol/g with 8% RSD (107 mg/g) and ranged between 1500 to 4000 μmol/g (50 and 125 mg/g) in diaphysis and was on average 2810 μmol/g with 14% RSD (87.1 mg/g) between 950 and 5700 μmol/g (22.0 and 175 mg/g) in epiphyses. There was a higher concentration of P in diaphysis than in epiphysis ($T = 9.54$, $P < 0.0001$, $DF = 119$), which is comparable with the finding for Ca concentration that shows that diaphysis were more mineralized than epiphyses ($T(119) = 12.2$, $P < 0.0001$). To investigate whether bone Pb was associated with a

reduction in bone mineral density, as has been reported in studies with other avian species (Gangoso *et al.*, 2009; Álvarez-Lloret *et al.*, 2014), I performed Spearman's correlation that revealed no statistical association between bone Ca concentration, as a surrogate of bone mineral contents, and bone Pb concentration for diaphysis (Figure 3.6A, Spearman's rho = 0.112, P = 0.192) or epiphyses (Figure 3.6B, Spearman's rho = 0.035, P = 0.570). There was also no statistical association between bone P concentration and bone Pb concentration in diaphysis (Figure 3.6C, Spearman's rho = 0.079, P = 0.357) or epiphyses (Figure 3.6D, Spearman's rho = 0.047, P = 0.444). To account for differences in bone mineral contents in each sample, I also normalized the bone Pb concentration by bone Ca content, and performed a Spearman's correlation that showed no statistical association between bone P concentration and Pb:Ca ratio for diaphysis (Figure 3.6E, Spearman's rho = 0.002, P = 0.984), but showed a significant negative association for epiphyses (Figure 3.6F, Spearman's rho = 0.157, P = 0.010).

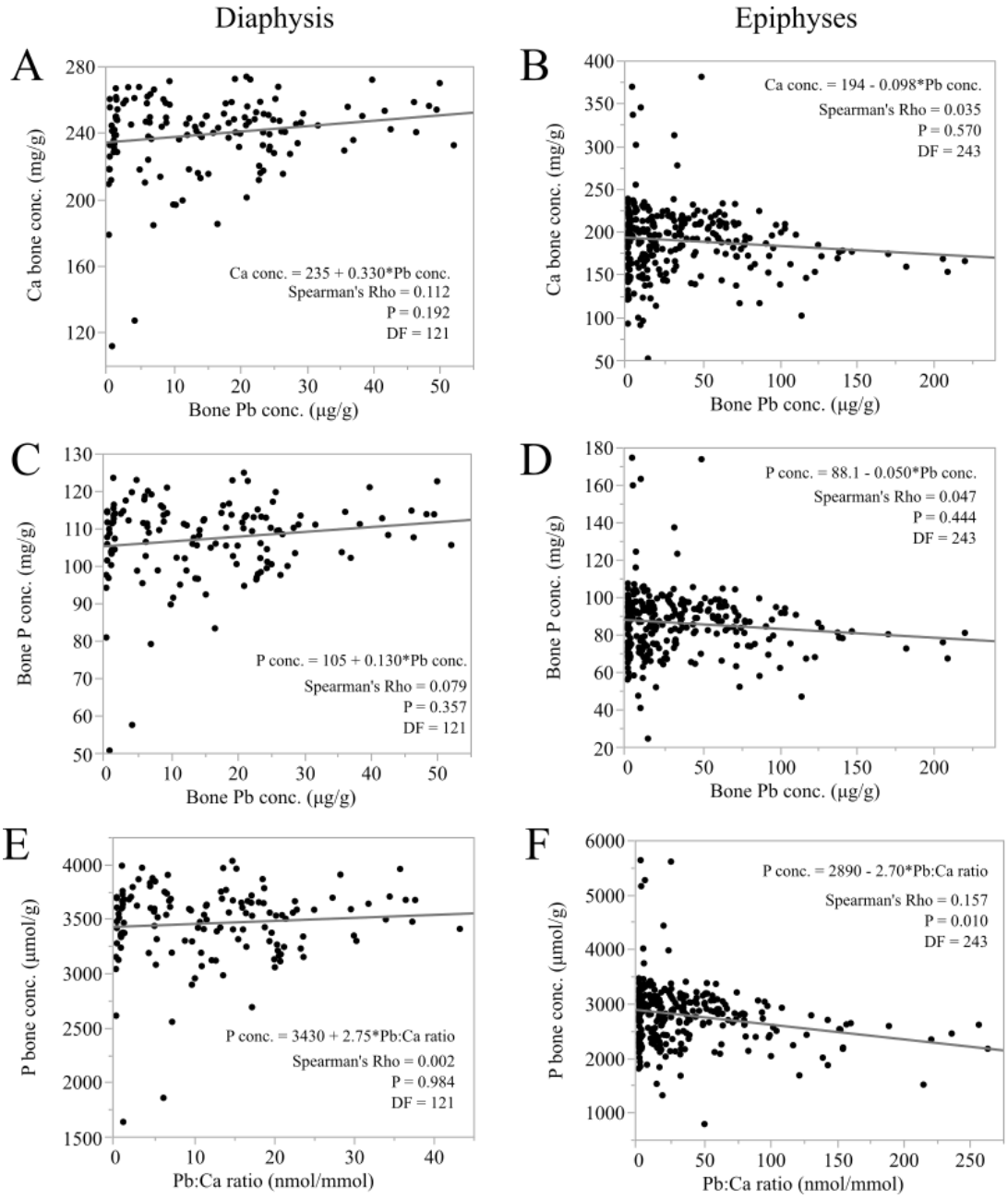


Figure 3.6 – Lead effect on bone mineral. (A-B) Bone Ca concentration vs bone Pb concentration. (C-D) Bone P concentration vs bone Pb concentration. (E-F) Bone P concentration vs bone Pb:Ca ratio. A, C, E show data for tibiotarsus diaphysis, while B, D, F show data for epiphyses (femur epiphysis distal and tibiotarsus epiphysis proximal). For each bone region, dots represent a single replicate measurement (n =

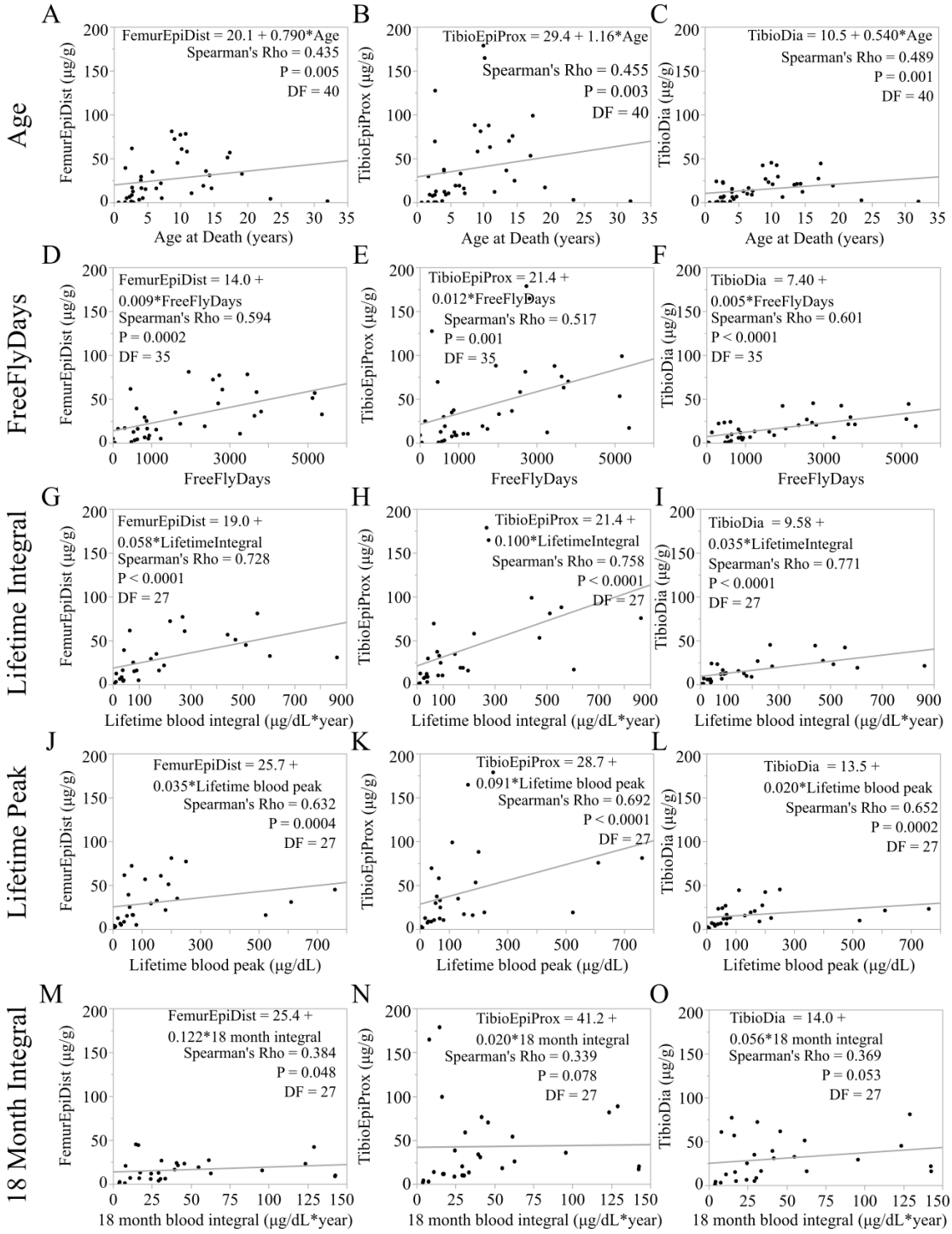
122 for diaphysis and n = 242 for epiphyses). The line represents the linear regression for the set of points, Spearman's Rho and P are noted in each figure.

3.3.4 Monitoring variables of cumulative Pb exposure

Trabecular bone regions (epiphyses) are believed to have higher turnover rates than mostly compact bone regions (diaphysis) in avian species (Hurwitz 1965), therefore, I expected that each bone region will reflect Pb exposure accumulated over different timeframes; for instance, tibiotarsus diaphysis is expected to reflect Pb exposure over the period of several months to years whereas Pb concentrations in epiphyses is expected to reflect Pb accumulation over the period of weeks to months. To determine which monitoring variables, measurable in free-flying condors, can be used to assess cumulative Pb exposure, I examined the correlations of bone Pb concentration in femur epiphysis distal, tibiotarsus epiphysis proximal and tibiotarsus diaphysis with seven monitoring variables: age, number of days free flying (FreeFlyDays), integral over time and peak of blood Pb concentration over the lifetime of a condor and in the 18 months prior to the condor's death, and proportion of days in the wild observed feeding on proffered carcasses with respect to the number of days free flying (FeedonProfferedCarcasses), that were found to be associated with survival rate and Pb mortality risk (Bakker *et al.*, 2016) (Table 3.6).

Significant associations were found between age and femur epiphysis distal (Spearman's Rho = 0.435, P = 0.005, DF = 35), tibiotarsus epiphysis proximal (Spearman's Rho = 0.455, P = 0.0028, DF = 35), and tibiotarsus diaphysis (Spearman's Rho = 0.489, P = 0.001, DF = 35) (Figure 3.7A-C). Number of free fly days was also significantly associated with femur epiphysis distal (Spearman's Rho = 0.594, P =

0.0002, DF = 35), tibiotarsus epiphysis proximal (Spearman's Rho = 0.517, P = 0.001, DF = 35), and tibiotarsus diaphysis (Spearman's Rho = 0.601, P < 0.0001, DF = 35) (Figure 3.7D-F). Other significant associations were found for lifetime blood Pb integral and peak, but not for 18 months blood Pb integral and peak (Table 3.6). To compare the slopes between bone Pb concentration with lifetime integral of blood Pb, number of free fly days, age and lifetime peak of blood Pb, an ANCOVA was performed. There was a significant difference in slopes between bone Pb concentration in each bone region and lifetime integral ($F(77) = 6.57$, $P < 0.0001$) and with number of free fly days ($F(101) = 8.40$, $P < 0.0001$), but not with age ($F(116) = 1.24$, $P = 0.29$) or lifetime peak ($F(1.71)$, $P = 0.14$). In the cases of number of free fly days and age, the slope with bone Pb concentration in tibiotarsus epiphysis proximal was significantly higher than with the other bone regions. Also, in the cases of number of free fly days and age, the slope with bone Pb concentration in tibiotarsus diaphysis was significantly lower than with the other bone regions. The significant difference in slope between tibiotarsus diaphysis and other bone regions for lifetime integral of blood Pb and free fly days supports the notion that both those variables are reflecting to some extent long term cumulative Pb exposure. Also, the lower slopes found for tibiotarsus diaphysis for lifetime integral of blood Pb and number of free fly days are consistent with Pb incorporation into tibiotarsus diaphysis being slower than the epiphyses.


A
FemurEpiDist = 20.1 + 0.790*Age
Spearman's Rho = 0.435
P = 0.005
DF = 40
FemurEpiDist (µg/g)
Age at Death (years)
B
TibioEpiProx = 29.4 + 1.16*Age
Spearman's Rho = 0.455
P = 0.003
DF = 40
TibioEpiProx (µg/g)
Age at Death (years)
C
TibioDia = 10.5 + 0.540*Age
Spearman's Rho = 0.489
P = 0.001
DF = 40
TibioDia (µg/g)
Age at Death (years)
Age
D
FemurEpiDist = 14.0 +
0.009*FreeFlyDays
Spearman's Rho = 0.594
P = 0.0002
DF = 35
FemurEpiDist (µg/g)
FreeFlyDays
E
TibioEpiProx = 21.4 +
0.012*FreeFlyDays
Spearman's Rho = 0.517
P = 0.001
DF = 35
TibioEpiProx (µg/g)
FreeFlyDays
F
TibioDia = 7.40 +
0.005*FreeFlyDays
Spearman's Rho = 0.601
P < 0.0001
DF = 35
TibioDia (µg/g)
FreeFlyDays
FreeFlyDays
G
FemurEpiDist = 19.0 +
0.058*LifetimeIntegral
Spearman's Rho = 0.728
P < 0.0001
DF = 27
FemurEpiDist (µg/g)
Lifetime blood integral (µg/dL*year)
H
TibioEpiProx = 21.4 +
0.100*LifetimeIntegral
Spearman's Rho = 0.758
P < 0.0001
DF = 27
TibioEpiProx (µg/g)
Lifetime blood integral (µg/dL*year)
I
TibioDia = 9.58 +
0.035*LifetimeIntegral
Spearman's Rho = 0.771
P < 0.0001
DF = 27
TibioDia (µg/g)
Lifetime blood integral (µg/dL*year)
Lifetime Integral
J
FemurEpiDist = 25.7 +
0.035*Lifetime blood peak
Spearman's Rho = 0.632
P = 0.0004
DF = 27
FemurEpiDist (µg/g)
Lifetime blood peak (µg/dL)
K
TibioEpiProx = 28.7 +
0.091*Lifetime blood peak
Spearman's Rho = 0.692
P < 0.0001
DF = 27
TibioEpiProx (µg/g)
Lifetime blood peak (µg/dL)
L
TibioDia = 13.5 +
0.020*Lifetime blood peak
Spearman's Rho = 0.652
P = 0.0002
DF = 27
TibioDia (µg/g)
Lifetime blood peak (µg/dL)
Lifetime Peak
M
FemurEpiDist = 25.4 +
0.122*18 month integral
Spearman's Rho = 0.384
P = 0.048
DF = 27
FemurEpiDist (µg/g)
18 month blood integral (µg/dL*year)
N
TibioEpiProx = 41.2 +
0.020*18 month integral
Spearman's Rho = 0.339
P = 0.078
DF = 27
TibioEpiProx (µg/g)
18 month blood integral (µg/dL*year)
O
TibioDia = 14.0 +
0.056*18 month integral
Spearman's Rho = 0.369
P = 0.053
DF = 27
TibioDia (µg/g)
18 month blood integral (µg/dL*year)
18 Month Integral

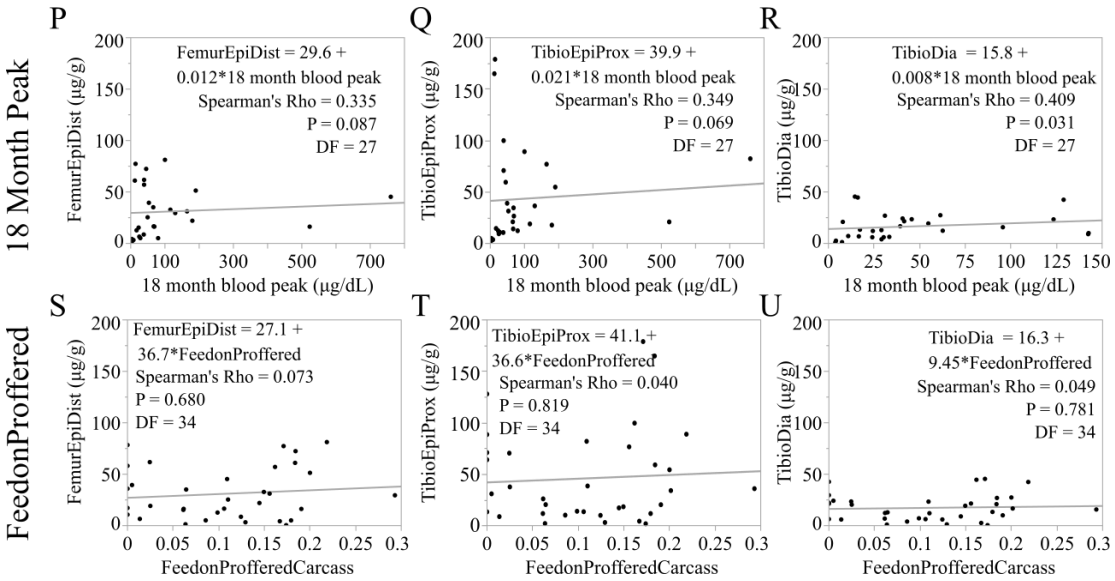


Figure 3.7 – Spearman's correlation between bone Pb concentrations in femur epiphysis distal, tibiotarsus diaphysis and tibiotarsus epiphysis proximal and monitoring variables related to Pb exposure (free flying days, lifetime integral of blood Pb concentration, proportion of days in the wild observed feeding on proffered carcass, age, lifetime peak blood Pb concentration, peak blood Pb concentration in the 18 months prior to death and integral of blood Pb concentration in 18 months prior to death). In each panel, each point represents a single condor, the line represents the linear regression (equation indicated in the panel), and the Spearman's correlation is also indicated. (A-C) show the relation between age in days and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis, respectively). (D-F) show the relation between number of free fly days and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis). (G-I) show the relation between the blood Pb lifetime integral and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis). (J-L) show the relation between blood Pb lifetime peak and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis). (M- O) show the relation between the blood Pb 18 months integral and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis). (P-R) show the relation between blood Pb 18 months peak and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis). (S-U) show the relation between proportion of days in the wild observed feeding on proffered carcasses and bone Pb concentration (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis).

I also checked the Spearman's correlation between each pair of monitoring variables, the results are summarized in Table 3.6. There was significant association

between age and number of free fly days, which was expected, since condors spend most of their lives in the wild. Also, the variables derived from blood Pb monitoring (integral and peak Pb over lifetime and over 18 months prior to death) were also well correlated.

Table 3.6 – Spearman's correlation matrix between bone Pb concentration in femur epiphysis distal, tibiotarsus diaphysis, tibiotarsus epiphysis proximal and monitoring variables: age, number of free fly days, integral and peak blood Pb concentration over condor's lifetime and 18 months prior to death, proportion of days observed in the wild feeding on proffered carcasses. First line in each cell is the Spearman's rho, while the P value statistical significance and number of birds (n) are shown in parentheses. Bold indicate significant relationships.

	Femur Epi Dist	**Tibio Epi Proxi**	**Tibiotarsus Diaphysis**	**Age at Death**	**Number of days free flying**	**Blood Pb Lifetime Integral**	**Blood Pb Lifetime Peak**	**Blood Pb 18 month Integral**	**Blood Pb 18 month Peak**
Tibio Epi Proxi	0.926 **(<0.0001, n = 41)**								
Tibiotarsus Diaphysis	0.945 **(<0.0001, n = 41)**	0.936 **(<0.0001, n = 41)**							
Age at Death	0.435 **(0.005, n = 36)**	0.455 **(0.003, n = 36)**	0.489 **(0.001, n = 36)**						
Number of days free flying	0.594 **(0.0002, n = 36)**	0.517 **(0.001, n = 36)**	0.601 **(<0.0001, n = 36)**	0.739 **(<0.0001, n = 36)**					
Blood Pb Lifetime Integral	0.728 **(<0.0001, n = 28)**	0.758 **(<0.0001, n = 28)**	0.771 **(<0.0001, n = 28)**	0.746 **(<0.0001, n = 28)**	0.798 **(<0.0001, n = 28)**				
Blood Pb Lifetime Peak	0.632 **(0.0004, n = 28)**	0.692 **(<0.0001, n = 28)**	0.652 **(0.0002, n = 28)**	0.544 **(0.003, n = 28)**	0.645 **(0.0002, n = 28)**	0.880 **(<0.0001, n = 28)**			

Blood Pb 18 month Integral	0.384 **(0.048, n = 28)**	0.339 (0.078, n = 28)	0.369 (0.053, n = 28)	0.144 (0.465, n = 28)	0.145 (0.461, n = 28)	0.506 **(0.006, n = 28)**	0.590 **(0.0009**, n = 28**)**		
Blood Pb 18 month Peak	0.335 (0.087, n = 28)	0.349 (0.069, n = 28)	0.409 **(0.031, n = 28)**	0.297 (0.125, n = 28)	0.343 (0.074, n = 28)	0.628 **(0.0003, n = 28)**	0.732 **(<0.0001, n = 28)**	0.893 **(<0.0001, n = 28)**	
Feed on Proof	0.073 (0.680, n = 36)	0.040 (0.819, n = 36)	0.049 (0.781, n = 36)	0.139 (0.427, n = 36)	0.098 (0.577 n = 36)	0.544 **(0.003, n = 28**)	0.470 **(0.012, n = 28)**	0.217 (0.268, n = 28)	0.340 (0.077, n = 28)

3.4 Discussion

This chapter addressed three main objectives. The first was to determine if bone Pb levels in femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis can be used as an additional piece of information to inform whether birds were Pb poisoned at the time of death. Bone Pb would be useful in cases where no soft tissue is available from the recovered carcass. The second was to determine whether cumulative Pb exposure, as reflected in bone Pb levels, was associated with monitoring and life history variables measured in free-flying birds, since long term cumulative Pb exposure is associated with sub-lethal effects (Smith and Flegal, 1992; Hu, Rabinowitz and Smith, 1998; Jendral *et al.*, 2008; Gangoso *et al.*, 2009; Álvarez-Lloret *et al.*, 2014). Finally, the third objective was to determine whether bone Pb levels were associated with a decrease in bone mineral content in condors. Since health effects due to Pb exposure have been reported to cause a reduction in bone mineral density (Gangoso *et al.*, 2009; Álvarez-Lloret *et al.*, 2014), I expected that a relationship between bone Pb and a reduction in bone mineral density would be found.

3.4.1 Bone Pb concentration as a biomarker of Pb exposure

For birds in the Non-exposed group, bone Pb concentrations were <2 μg/g (Figure 3.1A), depending on condor's age. With the exception of condor 32, bone Pb concentration was below 1 μg/g in all bones, which is comparable with the findings of Chapter 2 that showed that condors that were not exposed to elevated levels of Pb had bone Pb concentrations lower than 1 μg/g. Condor 32 is an older bird (32 years old, while the other four birds are younger than three years) which would allow for more

Pb accumulation in bones, even if at background levels. Data from condor 32 suggests that background levels appear to influenced by age, which is expected and comparable with finding in humans (Aufderheide and Wittmers, 1992; Wittmers *et al.*, 2008). Previous studies with bone Pb in avian species classify bone Pb concentration as "low" if it is below 10 μg/g (Thomas, Scheuhammer and Bond, 2009), bone Pb concentrations found in Non-exposed condors had 5-10-fold lower Pb concentrations.

Lead exposure history is assessed in condors through periodic monitoring of blood Pb (Finkelstein *et al.*, 2010, 2012). However, this monitoring depends on a condor being trapped in one of the field sites, which is not always possible in the desired semiannual periodicity. Even in the ideal case where a condor's blood Pb is measured twice in a year, only 10% of a condor's exposure history is assessed, considering that the half-life of Pb in blood is only ~13 days (Fry and Maurer, 2003). Therefore, a condor's history of Pb exposure is not well known if the bird was free flying. For the 41 condors analyzed in this study, half had an average of less than 3 blood Pb measurements per year, which would only be sufficient to cover ~15% of a condor's history of exposure (considering that the half-life of Pb in blood is of only ~13 days (Fry and Maurer 2003)). Moreover, 13 birds had less than one measurement a year, which would cover less than 5% of a condor's history of exposure. Even within each Pb exposure/CoD category, each condor has a unique exposure history, which makes the variability in bone Pb concentration between condors expected for those birds (Figure 3.1B-D). The variability in bone Pb concentration for the same bone region across birds observed in this study is comparable with findings in the literature that

show that bone Pb varies by 10-40-fold between birds living in the same geographic region (Thomas, Scheuhammer and Bond, 2009).

Birds in the Other CoD category were likely exposed to elevated amounts of Pb during the time they were free flying but were not poisoned at the time of death or at least were not diagnosed as having Pb toxicosis as their primary CoD. In fact, out of nine condors in this category, there is evidence of at least one event of Pb exposure for five condors (Table 3.1). Thus, the Pb burden found in the skeleton of Other CoD birds accumulated over months to years prior to the condor's death since there is no evidence of a Pb exposure event in the few weeks prior to death. Some Other CoD condors had higher bone Pb concentration than Pb CoD condors that were chelated in the two months prior to death or were younger than two years.

In Other Cod category condors that were not exposed to Pb in the few weeks prior to death (based on liver Pb <1 μg/g), Pb concentrations in tibiotarsus epiphysis proximal and femur epiphysis distal were statistically different than the tibiotarsus diaphysis. The differences in Pb concentrations can be explained by the fact that epiphyses are largely trabecular in composition and have higher mineral turnover than diaphysis, which are largely compact bones – as shown, e.g., in White Leghorn hens the turnover of trabecular bone is ~4% and only ~0.7% in compact bone per month (Hurwitz 1965). In humans, it has been shown that Pb accumulation in bone happens more prominently in diaphyses than in epiphyses over the lifetime (Wittmers *et al.*, 2008), which was not observed in Other CoD condors. However, due to lack of availability of older condors for this study, the effect seen here may be biased by the

relatively young age of the condors in this group (only three condors older than 18 years were available for use).

Birds in the Pb CoD group were Pb poisoned at the time of death. It is also likely that those condors were elevated exposed to Pb during their lifetime, in particular while free flying. Therefore, bone Pb concentration in those birds were due to a combination of effects between long term cumulative Pb exposure over the lifetime of each condor and the acute Pb exposure that was ultimately responsible for the condor's death. It is not possible to determine how much of the current skeletal Pb burden was from past compared to more recent exposures (Smith, Osterloh and Flegal, 1996). Since Pb concentration in tibiotarsus epiphysis proximal was higher than in femur epiphysis distal and that Pb CoD birds were exposed at the time of death, it is plausible that Pb incorporation in tibiotarsus epiphysis proximal is faster than in femur epiphysis distal. Given that Pb is incorporated into bone during bone formation (O'Flaherty, 1993), this is consistent with the suggestion that bone mineral turnover in tibiotarsus epiphysis proximal is faster than that of femur epiphysis distal. A study that evaluated bone Pb concentrations across the skeleton of eagles and swans showed that bone Pb concentrations were higher in mostly trabecular bones than bones with mainly compact bone (Ishii *et al.*, 2018).

The 2.6 – 5-fold difference in bone Pb concentration when comparing the same bone region across Other CoD and Pb CoD can be explained by the fact that birds in the Pb CoD category were exposed in the few days or weeks prior to death, whereas bird in the Other CoD were not and in bones with high turnover rate Pb concentrations

will rapidly change after an event of exposure and then decrease after the event ceased. Epiphysis is mostly composed of trabecular bone, while diaphysis is mostly composed of cortical bone (Clarke, 2008) and trabecular bone is known to have higher mineral turnover rate than compact bone (in White leghorn hens trabecular bone has ~4% turnover and compact bone has ~0.7% per month (Hurwitz 1965)). Also, the difference between the epiphyses in femur and tibiotarsus could be explained by the fact that tibiotarsus is non-pneumatized, that is, it has higher blood perfusion (Beaumont, 1968), which is known to increase the Pb uptake into bone (O'Flaherty, 1993).

The discriminant analysis results seem to suggest that bone Pb concentrations can be used to assess whether a condor was Pb poisoned at the time of death. Multiple studies report bone Pb as an indicator of Pb exposure (Pain, Sears and Newton, 1995; Mateo, Taggart and Meharg, 2003), however studies do not normally distinguish between bone and/or bone region used. Pb concentration in all three bones (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis) tested, was able to correctly classify more than 80% of birds (Figure 3.4). Each bone region had a different Pb concentration value as being indicative of Pb exposure. Given that the Pb concentrations in all the bone regions tested were well correlated (Figure 3.3 and Table 3.3, Spearman's rho > 0.92 with $P < 0.0001$ in all cases), it is not surprising that all bones presented the general same trend with regards to the discriminant analysis results. The three bone regions tested were also well correlated with liver Pb concentration, which also supports the high number of correctly classified condors in the discriminant analysis. Studies in waterfowl showed that bone Pb concentrations in tarsus (a mostly

compact bone) and liver Pb concentration were highly correlated ($R = 0.818$, $P < 0.001$ (Kim and Oh, 2014)).

The only condor that was consistently misclassified regardless of which bone region was condor 458. This condor was also misclassified when using a discriminant analysis with liver Pb concentration, indicating that it is an outlier with respect to the Pb CoD category. I have looked extensively at condor 458 and there is not a specific piece of data that can suggest why this condor is consistently misclassified. This bird was not chelated in the few months prior to its death. The bone Pb concentration is >5-fold higher than background, though it had lower Pb concentration within the Pb CoD group (among the four lowest Pb concentration for all bones). Liver Pb concentration (12 μg/g) was also higher than the cutoff considered diagnostic of Pb toxicosis (6 μg/g).

Among the condors that were classified differently depending on which bone was used, condor 125 was the only one misclassified by tibiotarsus diaphysis and condors 242 and 412 were the condors misclassified by femur epiphysis distal. Condor 125 was an older condor, having lived 19 years, which is one of the three oldest condors used in this study. Since Pb accumulates into compact bone over several years (Wittmers *et al.*, 2008) and tibiotarsus diaphysis is mostly composed of compact bone (Clarke, 2008), it is expected that using Pb concentration in diaphysis will be affected by the condor's age. In the case of condors 242 and 412, which were misclassified by femur epiphysis distal, there is not a clear explanation on why the classification disagrees depending on which bone was used. The confidence of the classification for those two cases is low (<60%, Table 3.4), indicating that those are borderline cases.

When using the discriminant analysis to predict to which group condors in the UNK CoD belong, it is not possible to have a final confirmation about the prediction of the discriminant analysis, since a definitive cause of death was not found for condors in the UNK CoD category. When considering Pb concentration of the tibiotarsus epiphysis proximal, three out of 12 birds in the UNK CoD category (25%) were predicted as Pb CoD (Figure 3.5 and Table 3.5). The proportion found here is comparable with the proportion (28%) of free flying adult condors found in a previous study to have died of Pb toxicosis (Rideout, 2012). When using the tibiotarsus diaphysis, five out of 12 condors in the UNK CoD category (41%) are predicted as Pb poisoned, considerably higher than the 28% reported in the literature (Rideout, 2012). The proportion of Pb poisoned birds found by tibiotarsus diaphysis suggests that the previous studies may have underestimated the number of condors that died of Pb toxicosis. The two condors that diverged in prediction between tibiotarsus epiphysis proximal and tibiotarsus diaphysis were 265 and 598.

Condors 102, 411, 499, 598 were chelated within 60 days of death, which could have an impact on how they were predicted by the model, since chelation reduces Pb burden in the blood. How chelation may affect bone Pb concentrations has not been largely explored in the literature. The effect of chelation is expected to have most impact bones with faster turnover rate, for instance tibiotarsus epiphysis proximal, which is mostly trabecular (Clarke, 2008), and least impact in bones with lower turnover rate, for instance tibiotarsus diaphysis, which is mostly compact (Clarke, 2008). Thus, it is hypothesized that in tibiotarsus diaphysis, bone Pb would not be as

reduced during chelation compared to epiphyses. For condor 411, this was not an issue, since it was predicted as Pb CoD regardless of which bone region was used. For the other three condors, chelation may have had altered the outcome of the prediction. However, condors 102 and 499 were both predicted as Other CoD, indicating that either they in fact did not die of Pb toxicosis, or the chelation treatment was effective in reducing the body Pb burden. Both those condors were chelated and then released back into the wild which makes it likely that the chelation was effective in reducing the Pb levels before release. Moreover, condor 598 – which was chelated in the three days prior to death – was classified as Pb CoD when using Pb concentration in tibiotarsus diaphysis and as Other CoD when using Pb concentration in tibiotarsus epiphysis proximal. The difference in classification depending on the bone used indicates that this condor may have been Pb poisoned and chelation affected the outcome of prediction when using tibiotarsus epiphysis proximal.

3.4.2 Monitoring variables of cumulative Pb exposure

Based on finding in humans, where Pb accumulates more in compact bone than trabecular bone over the lifetime (Aufderheide and Wittmers, 1992), it is expected that cumulative Pb exposure over several years in condors will be better associated with bones comprised mostly of compact bone, i.e., diaphysis. Based on the correlations found here, it seems that age (Figure 3.7A-C), number of free fly days (Figure 3.7D-F) and lifetime integral (Figure 3.7G-I) and peak of blood Pb (Figure 3.7J-L) are well suited to assess cumulative Pb exposure. A relation between bone Pb concentration in humerus and age was found (Pearson $R = 0.54$, $P = 0.013$, $n = 17$) in wild vultures

(Gangoso *et al.*, 2009) and it has been shown that bone Pb concentration differs ($P < 0.001$) between juveniles and adults red-legged partridge (*Alectoris rufa*) (Álvarez-Lloret *et al.*, 2014). An increase in bone Pb concentration in femur diaphysis with age was also reported in Spanish imperial eagles ($P < 0.001$, (Rodriguez-Ramos Fernandez *et al.*, 2011)). Condors are often trapped and kept in captivity for treatment, where Pb exposure is unlikely. Therefore, number of free fly days may better reflect the cumulative risk of Pb exposure for a condor than age. In fact the number of free fly days had a higher correlation with bone Pb concentration than age, since it only takes into account days where there was a chance of elevated Pb exposure (Table 3.6).

Blood Pb monitoring is far from ideal, as observed in another study that investigated the relationship between blood lead and survival (Bakker *et al.*, 2016). As a condor's blood Pb is measured approximately twice in a year, only 10% of the condor's exposure history is assessed (Fry and Maurer, 2003). Even with the limitations of blood Pb concentration measurements, lifetime integral of blood Pb concentration had the highest correlation with bone Pb concentration in tibiotarsus diaphysis). Integral of blood Pb is known to be a good indicator of cumulative Pb exposure, as indicated by correlation found in humans with blood Pb monitoring (Pearson's $R = 0.89$ between blood Pb integral and bone Pb concentration in tibiotarsus, Bergdahl et al. 1998). Lifetime blood Pb peak is also well correlated with bone Pb concentration in tibiotarsus diaphysis, since as a condor spends time in the wild, the likelihood of an event of exposure with increasing magnitude grows, making peak blood Pb concentration a proxy of cumulative Pb exposure.

3.4.3 Pb effect on bone mineral content

Bone Pb has been found to be associated with a reduction in bone mineral density in other avian species (Gangoso *et al.*, 2009; Naidoo *et al.*, 2012; Álvarez-Lloret *et al.*, 2014) and a reduction in mass of spleen in Argentinean wild ducks (Ferreyra *et al.*, 2015). Bone mineral content indicators (P and Ca concentrations) were evaluated against bone Pb indicators (Pb concentration and Pb:Ca ratio). The data presented here seem to suggest that, for epiphyses, there is a reduction in bone mineral with Pb exposure, as assessed by bone P concentration and by bone Pb:Ca atomic ratio (Figure 3.6F). No relation was found when considering bone Ca concentration and bone Pb concentration (Figure 3.6A-B) or between bone P concentration and Pb concentration (Figure 3.6C-D). The apparent difference in significance depending on which indicator is used for each quantity may be due the fact that the correlations between bone P and Ca and between bone Pb and Pb:Ca had some outliers, and even the small difference may be enough to bring the statistical results into significance. MicroCT results for two lead exposed condors and one Non-exposed condor showed a reduction in bone mineral density of 7-12% in tibiotarsus epiphysis proximal and a reduction of 1% in tibiotarsus diaphysis (Rizzi-Possignolo Unpublished Data). The MicroCT results seem to support the reduction in bone mineral contents found in this study.

When comparing with findings in the literature, one possibility for the low relationship found may be due to the relative low age of condors, compared to their natural lifespan, which may preclude condors to experience long term sub-lethal effects

of Pb exposure. A study with Argentinian wild ducks did not find an association between bone Pb and bone Ca and P or abnormalities in bone marrow (Ferreyra *et al.*, 2015). In any case, the data presented in Figure 3.6F seems to suggest that there is an effect of Pb exposure in reduction of bone mineral.

Reduction in bone mineral density is associated with an increase in the risk of fractures. In vultures exposed to Pb, a reduction in bone mineral and an increase in the occurrence of bone fractures was found (Gangoso *et al.*, 2009). Moreover, in humans a reduction in the bone mineral density was associated with an increase in the incidence (Barth, Williams and Kaplan, 1992) and a reduction in bone strength (Wachter *et al.*, 2002).

3.5 Conclusion

This chapter had three main findings. First, I demonstrated that bone Pb concentrations in femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis can can be used as an additional tool to help to inform whether a bird was Pb poisoned at the time of death. This prediction can be made with a single bone sample and tibiotarsus epiphysis proximal showed the highest prediction accuracy. Then, I have shown that age, number of free fly days and integral and peak blood Pb over the lifetime of a condor are associated with cumulative Pb exposure, as reflected in Pb levels in tibiotarsus diaphysis. Finally, my data suggest that there seems to be a relationship between bone Pb and a reduction in bone mineral contents, which can increase the likelihood of bone fractures and have impacrs on condor survival, but the data presented here were not conclusive. The findings from this chapter can help

improve the monitoring of Pb poisoning in condors and other large avian species, such raptors. The association with monitoring variables may be particularly useful to provide additional tools to assess sub-lethal Pb exposure that are related with negative health outcomes.

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Chapter 4) Investigate lead uptake into condor bones using stable lead isotopes

4.1 Introduction

Bone lead (Pb) concentrations are known to vary between bones and within a bone in humans (Wittmers *et al.*, 1988; Hu, 1998) and avian species (Ishii *et al.*, 2018). The differences in Pb concentrations are thought to be associated with bone mineral turnover rates (Rabinowitz, 1991; O'Flaherty, 1993). For avian species, Pb accumulation is expected to behave differently than Pb accumulation in mammal bones as some avian bones are pneumatized (Cubo and Casinos, 2000), which may affect bone perfusion. Bone perfusion is known to affect Pb uptake into bone (O'Flaherty, 1993).

In Chapters 2 and 3 of this dissertation, I discussed the use of bone Pb concentration as a biomarker of Pb exposure. Lead concentrations varied across bone regions in condors that died of Pb poisoning, for instance bone Pb concentration was on average 3-fold higher in tibiotarsus epiphysis proximal than in tibiotarsus diaphysis. As a consequence, the assessment of Pb exposure history may vary depending on which bone region was selected as an indicator of Pb exposure. In Chapter 3, I examined the utility of using bones as a marker for acute and chronic lead exposure in avian species. I presented discriminant analyses results using multiple bone Pb indicators and found that bone Pb levels, in particular in the tibiotarsus epiphysis proximal, can help inform a condor's lead status at time of death. I also found that bone Pb indicators, in particular

in tibiotarsus diaphysis, are associated with age, time in the wild, and lifetime integral of blood Pb concentration.

Even though some studies in birds measured Pb concentration in multiple bones (Mateo, Taggart and Meharg, 2003; Gangoso *et al.*, 2009; Ishii *et al.*, 2018), there are currently no studies in avian species that have quantified the Pb uptake rate into multiple bone regions. In humans, stable Pb isotopic composition analyses has been used to determine Pb kinetics within the body by assessing relative contributions from different endogenous and exogenous sources, in particular from the skeleton into the bloodstream (Rabinowitz, 1991; Smith, Osterloh and Flegal, 1996; Gwiazda, Campbell and Smith, 2005). In primates, controlled exposure to Pb with known isotopic composition was used to determine historical Pb contribution into blood and bones and found that trabecular portion of tibia incorporated Pb 2.5-fold faster than compact portion of tibia (Inskip *et al.*, 1996).

Existing methods to determine Pb uptake rates into bones rely on measuring the magnitude and isotopic composition of Pb exposure (Inskip *et al.*, 1996; Smith, Osterloh and Flegal, 1996; Franklin *et al.*, 1997). Such methods require that exposure sources possess distinct isotopic compositions compared to the isotopic composition of endogenous lead already in the body (Rabinowitz, 1991; Inskip *et al.*, 1996; Smith, Osterloh and Flegal, 1996). In wild animals that are exposed to Pb, it is not possible to measure blood Pb as frequently as necessary to apply existing methods to determine Pb uptake into bone. Segmented feather analysis provides a good surrogate for blood

Pb over different timeframes (Church *et al.*, 2006; Finkelstein *et al.*, 2010) and can be used as a proxy for blood Pb over the time frame of feathers growth.

The main objective of this chapter was to use Pb stable isotopic composition ($^{207}Pb/^{206}Pb$ ratio) to estimate Pb incorporation rate into bone during an exposure event. Lead stable isotope tracers can be used to determine incorporation rate into bone by estimating the percentage contribution attributed to a Pb exposure event. For wild condors, segmented feather analysis can be used to assess the timing and magnitude of Pb exposure events over the few months timeframe of feather growth prior to death. Also, the feather segment Pb stable isotopes can be used o infer the source of exposure.

The model employed in this work is based on a two endmember mixing model previously used in humans (Smith, Osterloh and Flegal, 1996) and similar to the model employed in nonhuman primates (Inskip *et al.*, 1996). I used liver, growing feather and bones (tibiotarsus epiphysis proximal, femur epiphysis distal, and tibiotarsus diaphysis) to estimate lead uptake rates. Lead is transferred to other tissues from the bloodstream. A fully grown feather is not a metabolically active tissue, as Pb is incorporated into the feather tissue only during growth. Thus, it is possible to estimate pre- and post-exposure blood Pb concentrations and isotopic composition by segmented feather analysis if the exposure event occurred during feather growth. Bones and liver are dynamically active metabolic tissues with constant blood flow and thus exchange Pb with the bloodstream (Figure 4.1). I expect that following an exposure event, liver will uptake Pb faster than bones, and that among bones, tibiotarsus epiphysis proximal will have the faster Pb uptake than regions comprised primarily of compact bone, since largely trabecular

epiphyses have faster mineral turnover rate (Hurwitz, 1965; Clarke, 2008). Moreover, in condors, tibiotarsus is a non-pneumatized bone while femur is pneumatized. Pneumatization may reduce blood perfusion. Blood perfusion is associated with Pb uptake into bone (O'Flaherty, 1993).

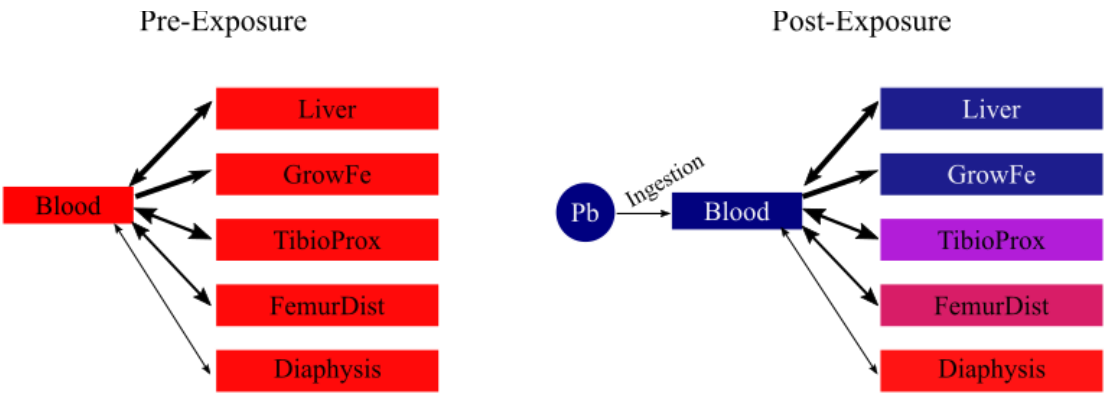

Figure 4.1 – Schematic representation of expected Pb uptake following an event of Pb exposure. The condor body pre- and post- exposure are represented in the left and right figure respectively. The tissues used in this work are represented. Arrows represent the Pb exchange between tissues, and arrow thickness represent hypothetical rates at which Pb is exchanged. Box colors represent hypothetical isotopic compositions in each tissue and boxes do not reflect the amount of Pb in each tissue. Pre-exposure, all tissues are expected to present the same isotopic signature, and post-exposure, isotopic signature is expected to change at a rate proportional to the exchange/accumulation rate.

4.2 Methods

4.2.1 Overall approach and sample selection

To assess Pb uptake into different bone regions of California Condors, I used Pb stable isotope ($^{207}Pb/^{206}Pb$) analysis of bones (femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis), liver, and segmented feathers. Following an exposure event, differences in $^{207}Pb/^{206}Pb$ ratio between the skeletal and circulatory Pb may occur because of the long residency times of Pb in bones and since

bone Pb is a mixture of Pb from past and current exposure (Smith, Osterloh and Flegal, 1996).

Segmented feather analysis of feathers growing at the time of death were used to provide a history of Pb exposure over the 2 – 4 month period that spans feather growth (Finkelstein *et al.*, 2010). This analysis provides the source of Pb exposure from stable Pb isotopic composition, a timeline, and the magnitude of exposure (Finkelstein *et al.*, 2010). Since the fully-grown vane of feather is not a living tissue and there is no Pb exchange from feather to blood, feather Pb is a surrogate for blood Pb at the time the feather material was formed.

To determine the relative contribution from a Pb exposure to each tissue (liver and bone regions), I used the following mixing model formula:

$$\%\ \text{Pb from exposure}_{\text{source}} = 100 - \frac{\text{PbIC}_{\text{post-exposure}} - \text{PbIC}_{\text{exposure}}}{\text{PbIC}_{\text{pre-exposure}} - \text{PbIC}_{\text{exposure}}} \text{ x } 100, \qquad (1)$$

where $\%\ Pb\ from\ exposure_{source}$ is the percent of Pb in the measured tissue that can be attributed to the exposure source, $PbIC_{post-exposure}$ is the $^{207}Pb/^{206}Pb$ measured in each tissue, $PbIC_{pre-exposure}$ is the $^{207}Pb/^{206}Pb$ ratio in the tissue before the exposure event, and $PbIC_{exposure}$ is the $^{207}Pb/^{206}Pb$ ratio of the exposure source. In cases where $^{207}Pb/^{206}Pb$ ratio post-exposure is higher than pre-exposure or lower than exposure $^{207}Pb/^{206}Pb$ ratio, the percent Pb attributed to exposure may be less than zero or greater than 100%, in those cases, I reported zero and 100% respectively.

Samples were selected based on cases of condors that were either not exposed to elevated amounts of Pb or had Pb poisoning as diagnosed cause of death, in addition to: 1) availability of bone samples: femur epiphysis distal, tibiotarsus diaphysis and,

tibiotarsus epiphysis proximal; 2) availability of growing feather sample; 3) availability of liver sample. Based on this selection criteria, ten condors were selected for analysis. Condors 445 and 502 lived in captivity their entire life, and condors 112, 238, 245, 286, 306, 318 345, and 458 lived in the wild and were diagnosed to have died from Pb toxicosis (Table 4.1).

Table 4.1 – Description of cases used in this study. Each sample is uniquely identified by a studbook number, which is also routinely used for monitoring during the lifetime of the bird.

Studbook #	Age (years)	Sex	Free Fly Days[i]	Pathologist CoD[ii]	Last known chelation before death (days)[iii]
445	2.9	Male	0	Euthanasia	None
502	2.8	Male	0	Conspecific trauma	None
112	17.0	Female	5125	Pb toxicosis	5 days
238	7.1	Male	1227	Pb toxicosis	3 days
245	6.3	Female	635	Pb toxicosis	14 days
286	7.0	Male	1729	Pb toxicosis	68 days
306	10.1	Female	2809	Pb toxicosis	No info
318	9.5	Male	2702	Pb toxicosis	17 days
345	9.0	Male	2568	Pb toxicosis	No info
458	2.8	Female	527	Pb toxicosis	No info

[i] Number of free flying days is the total number of days that a condor was in the wild during its lifetime.
[ii] Condors recovered dead undergo routine necropsy by a board-certified pathologist to determine the cause of death.
[iii] Chelation is a chemical treatment that helps reduce a condor's lead levels. Chelation treatment was typically administered to condors when blood Pb levels higher than 35 μg/dL were measured during routine monitoring (USFWS, 2014). I report the number of days between the start of the chelation and death.

4.2.2 Sample collection, processing and analysis

Bone samples

Bone samples were collected from femur epiphysis distal, tibiotarsus epiphysis proximal, and tibiotarsus diaphysis, processed and processed for analyses in a high-efficiency particulate attenuated (HEPA) filtered air laboratory using established trace metal clean techniques, as described elsewhere (Smith et al. 1996; Finkelstein et al. 2003; Finkelstein et al. 2010). Briefly, bone samples were collected using a titanium corer fitted to a power drill, or Dremel saw fitted with a stainless steel blade. Bone

samples (50 – 100 mg) were cleaned of adhering soft tissue with a stainless steel scalpel, rinsed with ultrapure grade water (Milli-Q system from Millipore, Inc), acetone and ultrapure 5% HNO_3 acid. Subsamples were then dried in an oven for 3 days (65 °C) and weighed. Duplicate subsamples were collected from each bone location. Bone samples were digested in 1 mL ultrapure concentrated HNO_3 in polytubes for 6 hours in a water bath (80°C); after digestion, samples were cooled for ~30 min. and 5 mL of ultrapure MQ water was added to dilute the samples.

Growing feather and liver

As previously reported by Church et al., 2006 and Finkelstein et al., 2010, growing feathers were collected post mortem and, individual sections of feather vane (~2 cm width along rachis axis) were treated as separate samples; each feather section was processed under trace metal clean conditions to remove surface contamination by washing sequentially with 1% HNO_3, ultrapure water, acetone, and ultrapure water, then they were dried in over at 65 °C overnight, and weighed. Condor feathers grow at a rate of about 4.4 ± 0.39 mm/day and, in a growing feather, the most proximal feather vane sample that is available for collection reflects a condor's blood ~10 – 20 days before the collection date (Finkelstein *et al.*, 2010). In this work, I segmented feathers at approximately every 2 cm, therefore, each segment corresponds to ~4.5 ± 0.41 days of growth. The relationship between blood lead (µg/dL) and feather lead (µg/g) concentrations (i.e., blood lead:feather lead ratio) is ~19:1 (Finkelstein et al., 2010), and was used to estimate the blood Pb level based on the measured feather Pb level.

Liver was processed as described previously (Finkelstein et al., 2003, 2010; Gwiazda et al., 1998; Smith et al., 1996); briefly, livers were sub-sampled to around 250 mg wet-weight, rinsed in sequence with 1% HNO_3, ultrapure water, then were dried for three days in over at 65 °C, weighted, and digested overnight in 2 mL sub-boiling concentrated HNO_3 in closed Teflon vials, evaporated to dryness, and reconstituted in 5% HNO_3 for analysis.

ICP-MS Analysis

As previously described by Finkelstein et al., 2003 and Gwiazda et al., 1998, sample lead concentrations and isotope ratios were determined by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Element XR magnetic sector), measuring masses of ^{206}Pb and ^{207}Pb. ^{205}Tl was used as an internal standard. The precision of the $^{207}Pb/^{206}Pb$ isotope ratio measurements was ~0.2% (2x the relative standard deviation, 2RSD), based on condor tissue samples analyzed in triplicate within an analytical run. Between-run measurement precision was <0.2% (2x the relative standard deviation, 2RSD) between the two runs done over the period of a couple weeks, based on repeated measurements of laboratory standard blood sample. Isotope ratios ($^{207}Pb/^{206}Pb$) that differed by <0.2% (i.e., the long-term measurement precision) were considered measurably indistinguishable.

Data Analysis

Bone samples were analyzed in duplicate (Appendix B Table S.3). For the mixing model calculations, bone Pb concentrations were reported as the average of duplicates for each bone region, the percentage difference between bone Pb

concentration duplicates ranged between 2% and 36% (Table 4.2). The $^{207}Pb/^{206}Pb$ reported for bone samples was also the average of duplicates, the percentage difference between duplicates ranged between 0.01% and 0.17% (Table 4.2).

Table 4.2 – Average of bone Pb concentration and $^{207}Pb/^{206}Pb$ for each condor and each bone region as well as percentage difference of duplicates. Individual replicate $^{207}Pb/^{206}Pb$ values in Appendix B Table S.3 and Pb concentration in Appendix A Table S.1.

Studbook #	**Bone Region**	**Bone Pb conc. (µg/g)**	**Bone Pb conc. % diff[i]**	**Bone $^{207}Pb/^{206}Pb$**	**Bone $^{207}Pb/^{206}Pb$ % diff**
445	Femur epiphysis distal	0.100	11%	0.8272	0.13%
445	Tibiotarsus epiphysis proximal	0.121	5%	0.8227	0.17%
445	Tibiotarsus diaphysis	0.082	7%	0.8229	0.14%
502	Femur epiphysis distal	0.404	14%	0.8314	0.09%
502	Tibiotarsus epiphysis proximal	0.339	5%	0.8338	0.01%
502	Tibiotarsus diaphysis	0.227	7%	0.8247	0.05%
112	Femur epiphysis distal	51.4	8%	0.8131	0.07%
112	Tibiotarsus epiphysis proximal	53.5	11%	0.8150	0.15%
112	Tibiotarsus diaphysis	27.4	6%	0.8147	0.01%
238	Femur epiphysis distal	5.11	14%	0.8462	0.02%
238	Tibiotarsus epiphysis proximal	10.6	36%	0.8378	0.17%
238	Tibiotarsus diaphysis	13.4	25%	0.8441	0.16%
245	Femur epiphysis distal	16.3	2%	0.8336	0.16%

245	Tibiotarsus epiphysis proximal	19.3	7%	0.8339	0.17%
245	Tibiotarsus diaphysis	10.1	22%	0.8293	0.16%
286	Femur epiphysis distal	22.0	34%	0.8173	0.07%
286	Tibiotarsus epiphysis proximal	16.1	16%	0.8170	0.07%
286	Tibiotarsus diaphysis	9.03	4%	0.8179	0.01%
306	Femur epiphysis distal	53.4	31%	0.8197	0.01%
306	Tibiotarsus epiphysis proximal	161	24%	0.8176	0.01%
306	Tibiotarsus diaphysis	14.9	12%	0.8235	0.07%
318	Femur epiphysis distal	41.3	13%	0.8351	0.23%
318	Tibiotarsus epiphysis proximal	98.0	24%	0.8302	0.17%
318	Tibiotarsus diaphysis	22.0	6%	0.8303	0.09%
345	Femur epiphysis distal	72.4	27%	0.8215	0.10%
345	Tibiotarsus epiphysis proximal	58.3	8%	0.8211	0.03%
345	Tibiotarsus diaphysis	26.9	7%	0.8215	0.13%
458	Femur epiphysis distal	12.7	13%	0.8207	0.07%
458	Tibiotarsus epiphysis proximal	12.9	19%	0.8205	0.11%
458	Tibiotarsus diaphysis	7.16	30%	0.8209	0.09%

[i] Percentage difference calculated as (a-b)/b*100%.

4.3 Results

4.3.1 Overall approach

Figure 4.2 shows a hypothetical case, with an ideal profile of exposure to illustrate how the mixing model was formulated based on clear pre-exposure and exposure conditions. The ideal case assumes the $^{207}Pb/^{206}Pb$ ratio of the tissues before exposure can be estimated from the $^{207}Pb/^{206}Pb$ ratio of the feather segments pre-exposure. It also assumes that the $^{207}Pb/^{206}Pb$ ratio of the exposure source can be estimated from the feather $^{207}Pb/^{206}Pb$ ratio at the peak of exposure. In the ideal case, the pre-exposure $^{207}Pb/^{206}Pb$ ratio in feather is 0.8450, with background Pb concentration (~0.3 μg/g). There is a change in the feather $^{207}Pb/^{206}Pb$ ratio that coincides with an increase in feather Pb concentration. At the peak feather Pb concentration, the $^{207}Pb/^{206}Pb$ ratio is 0.8028, which is assumed to reflect the $^{207}Pb/^{206}Pb$ ratio of the exposure source. Post-exposure, the bone $^{207}Pb/^{206}Pb$ ratio is intermediate between the pre- and post-exposure $^{207}Pb/^{206}Pb$ ratio. The proportion of Pb in bone that can be attributed to the exposure source will depend on the Pb uptake rate of each bone region, the relative magnitude and duration of the Pb exposure event and cumulative exposure of the condor. The bone Pb uptake rate is expected to be influenced by the bone perfusion rate and metabolic activity, which may be influenced by bone and bon type. In this ideal case, the percent of Pb in liver attributed to the exposure event that occurred 58 days before death was 100%, in tibiotarsus epiphysis proximal was 60%, in femur epiphysis distal was 35% and in tibiotarsus diaphysis 12%. The $^{207}Pb/^{206}Pb$ ratio in each tissue reflects the $^{207}Pb/^{206}Pb$ ratio of the exposure source

depending on the tissue Pb uptake rate, magnitude of exposure, and time elapsed between the exposure and death.

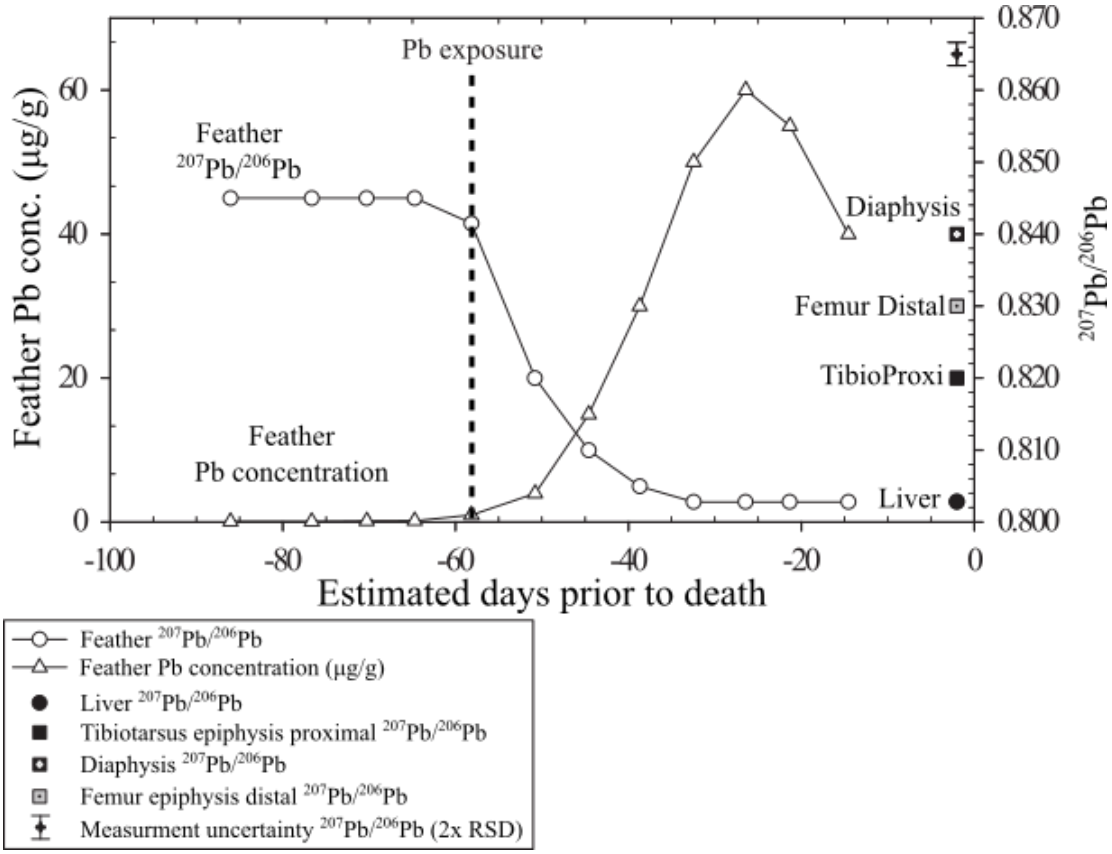

Figure 4.2 – Hypothetical case, with a single event of exposure that occurred 58 days prior to death. Liver $^{207}Pb/^{206}Pb$ ratio is measurably different than that of bones and the $^{207}Pb/^{206}Pb$ ratio pre-exposure is different than the $^{207}Pb/^{206}Pb$ ratio post-exposure. The pre-exposure $^{207}Pb/^{206}Pb$ ratio in this case is 0.8450, the exposure $^{207}Pb/^{206}Pb$ ratio reflects the peak of Pb concentration in the feather and is 0.8028. The $^{207}Pb/^{206}Pb$ ratio measurement error (±2x RSD) is represented by the error bar in the top right corner of the figure.

4.3.2 Selected cases

To assess Pb uptake into bone, I selected cases that had a measurable change in $^{207}Pb/^{206}Pb$ ratio between multiple segments of feather, which is indicative of changes

in a Pb exposure source over the timeframe of the feather growth. Condors were selected to estimate bone Pb uptake rates, based on the following criteria:

1) Feather Pb and $^{207}Pb/^{206}Pb$ ratio data provided evidence of a single Pb exposure event over the period of feather growth, going from low feather Pb to high feather Pb, with a measurable change in feather $^{207}Pb/^{206}Pb$ ratio (i.e., > ± measurement error, 0.2% RSD);
2) The liver $^{207}Pb/^{206}Pb$ ratio was measurably different than bone $^{207}Pb/^{206}Pb$ ratio in at least one bone.

In cases where a lead fragment was available, the fragment $^{207}Pb/^{206}Pb$ ratio was used as the exposure $^{207}Pb/^{206}Pb$, otherwise, the feather $^{207}Pb/^{206}Pb$ at the peak of feather Pb concentration was used as the exposure $^{207}Pb/^{206}Pb$. The pre-exposure $^{207}Pb/^{206}Pb$ ratio was selected as the feather $^{207}Pb/^{206}Pb$ ratio pre-exposure or the femur epiphysis distal $^{207}Pb/^{206}Pb$ ratio. Femur epiphysis distal was used since for condor 238 it had the same value as pre-exposure feather $^{207}Pb/^{206}Pb$ ratio.

The cases that fit the criteria discussed above were 238, 306, and 318 (Table 4.3). Condors 445 and 502 lived in captivity for their whole life and were likely not exposed to elevated amounts of Pb (Table 4.3). For condors 112, 286, and 458, the liver $^{207}Pb/^{206}Pb$ ratio was not measurably different than any of the bone $^{207}Pb/^{206}Pb$ ratios, and condors 245 and 345 had two exposure events during the timeframe of the feather growth (Table 4.3). Therefore, condors 112, 245, 286, 345, 445, 458, and 502 did not fit the criteria to estimate to measure Pb uptake rates.

For the condors that fit the criteria (238, 306, and 318), feather segments were selected to represent two distinct phases during the 7 to 13 weeks period of feather growth, one phase before the exposure started and one during/after the exposure. To identify the timing of the exposure event, I relied on changes in the $^{207}Pb/^{206}Pb$ ratio in the feather.

There are key assumptions used in this analysis. The $^{207}Pb/^{206}Pb$ ratio of the exposure source was measured from the ammunition recovered from the bird or estimated from feather at peak exposure. Pre-exposure $^{207}Pb/^{206}Pb$ of bone and liver tissues was estimated from the $^{207}Pb/^{206}Pb$ ratio of the pre-exposure feather segments or femur epiphysis distal. However, the pre-exposure feather may not accurately represent the pre-exposure $^{207}Pb/^{206}Pb$ ratio of the bones and liver.

Table 4.3 – Selection criteria and data sources for cases from the 10 birds analyzed to be used in the model and whether each condor meets each criterion. Shaded lines indicate condors that fit both criteria.

Studbook #	Feather Pb concentration and $^{207}Pb/^{206}Pb$ ratio data provide evidence of single Pb exposure event over period of feather growth[i]	Liver $^{207}Pb/^{206}Pb$ ratio is measurably different than bone $^{207}Pb/^{206}Pb$ ratio	Ammo was available for analysis[ii]
238	Yes	Yes	No
306	Yes	Yes	No
318	Yes	Yes	Yes
445	No exposure	Yes	No
502	No exposure	Yes	No
112	Yes	No	No
245	Two events of exposure	Yes	No
286	Yes	No	No
345	Two events of exposure	No	No
458	Yes	No	No

[i] Reflected in the feather, Pb concentration changes from background Pb level (~0.3 µg/g) to significantly above background (>1.5 µg/g, which is indicative of clinical exposure), with a measurable change in feather $^{207}Pb/^{206}Pb$ ratio (i.e., >±2x the measurement error).

[ii] In cases where a Pb fragment was recovered from GI tract, the ammunition $^{207}Pb/^{206}Pb$ ratio was used as the exposure source.

4.3.3 Lead uptake rate into condor bone and liver

Condor 238: Feather analysis of condor 238 revealed an event of exposure starting 58 days prior to death, where feather Pb concentration increased from 1 to ~6 µg/g. The estimated blood Pb concentration during the exposure event was ~114 µg/dL (assuming a relationship between blood lead (µg/dL) and feather lead (µg/g) concentrations of ~19:1, Finkelstein et al 2010). The $^{207}Pb/^{206}Pb$ ratio at the peak of the exposure was 0.8028, lower than background (~0.8363, M. E. Finkelstein et al. 2012).

From the beginning of the timeframe captured by the feather growth until 58 days prior to death (at the time of the exposure event), feather Pb concentration was ~0.1 – 0.2 µg/g (Figure 4.3), below the background level for Pb concentration in feather (0.3 µg/g, Finkelstein et al. 2010), and $^{207}Pb/^{206}Pb$ ratio was 0.8402.

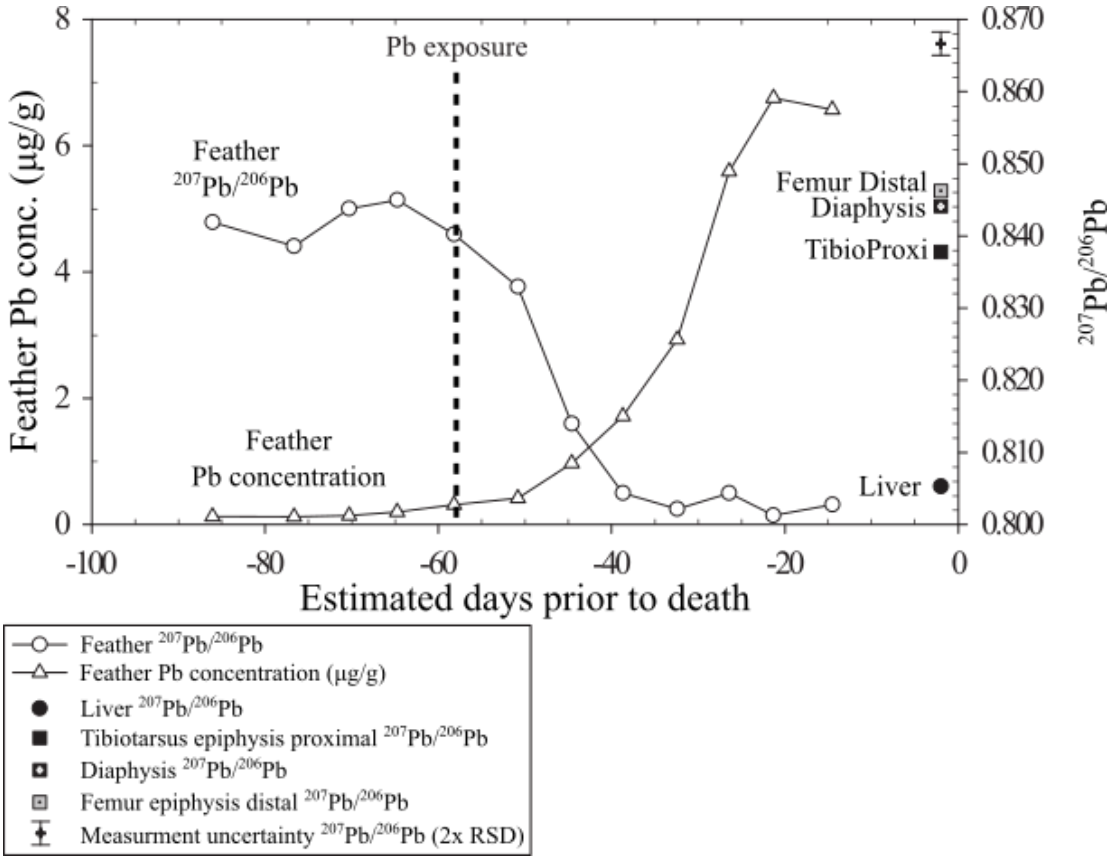

Figure 4.3 – Segments from condor 238 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. The dashed line marks the event of Pb exposure 58 days prior to death. $^{207}Pb/^{206}Pb$ ratio for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure. The Pb concentration in femur

epiphysis distal was 5.11 µg/g, in tibiotarsus diaphysis was 13.4 µg/g, and in tibiotarsus epiphysis proximal was 10.6 µg/g. Liver Pb concentration was 1.89 µg/g.

Condor 306: For condor 306, the feather analysis revealed an event of exposure starting 55 days prior to death. The peak feather Pb concentration was 65 µg/g (estimated blood Pb concentration of 1240 µg/dL) with $^{207}Pb/^{206}Pb$ ratio of 0.8154. From around 90 to 55 days prior to death, feather Pb concentration was ~1-2 µg/g and $^{207}Pb/^{206}Pb$ ratio was ~0.8242 (Figure 4.4).

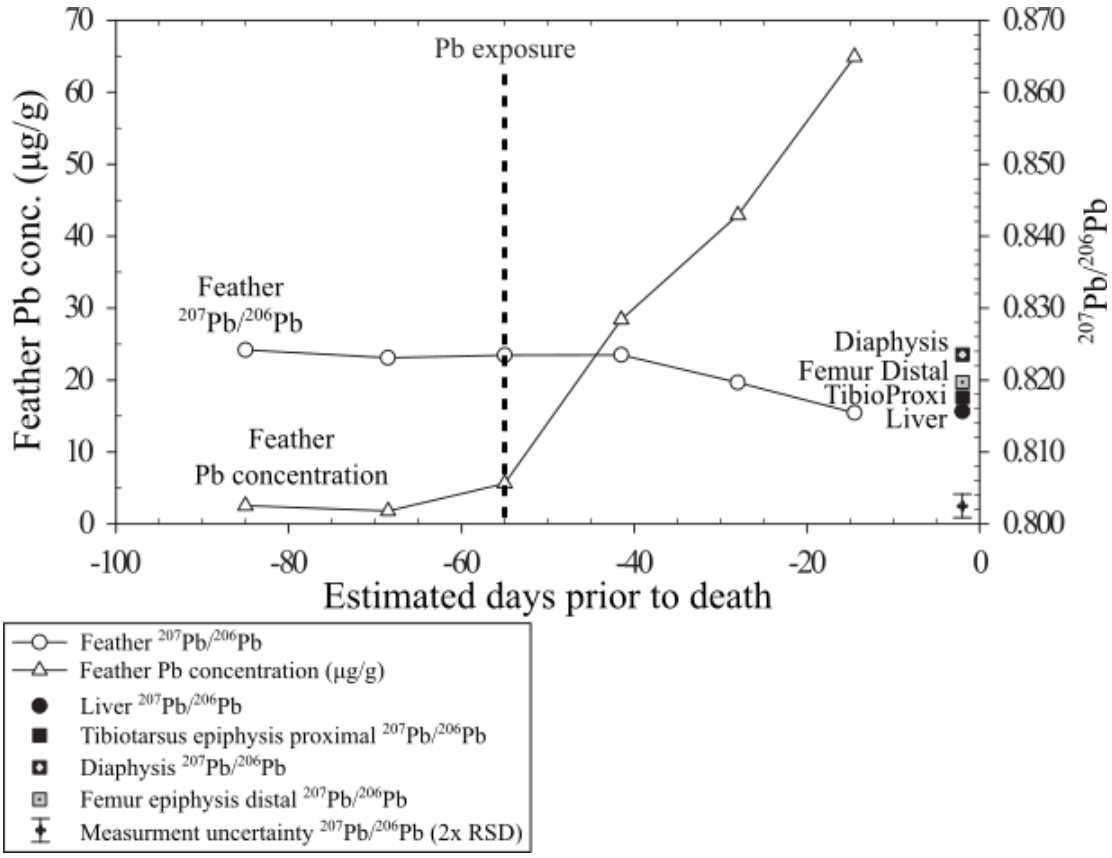

Figure 4.4 – Segments from condor 306 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and feather was collected post-mortem. The dashed line marks the event of Pb exposure that occurred 55 days prior to death. $^{207}Pb/^{206}Pb$ ratio for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is

represented by the error bar in the bottom left corner of the figure. The Pb concentration in femur epiphysis distal was 61.1 µg/g, in tibiotarsus diaphysis was 20.9 µg/g, and tibiotarsus epiphysis proximal was 165 µg/g. Liver Pb concentration was 208 µg/g.

Condor 318: For condor 318, there was an increase in feather Pb concentration (Figure 4.5) from the oldest feather segment (~50 days prior to death) until ~25 days prior to death, from ~21 µg/g to ~58 µg/g, which indicates that the Pb exposure event started prior to the timeframe of feather growth. Then, feather Pb concentration dropped to ~32 µg/g around 15 days prior to the condor's death. The feather $^{207}Pb/^{206}Pb$ ratio was between 0.8254 and 0.8287.

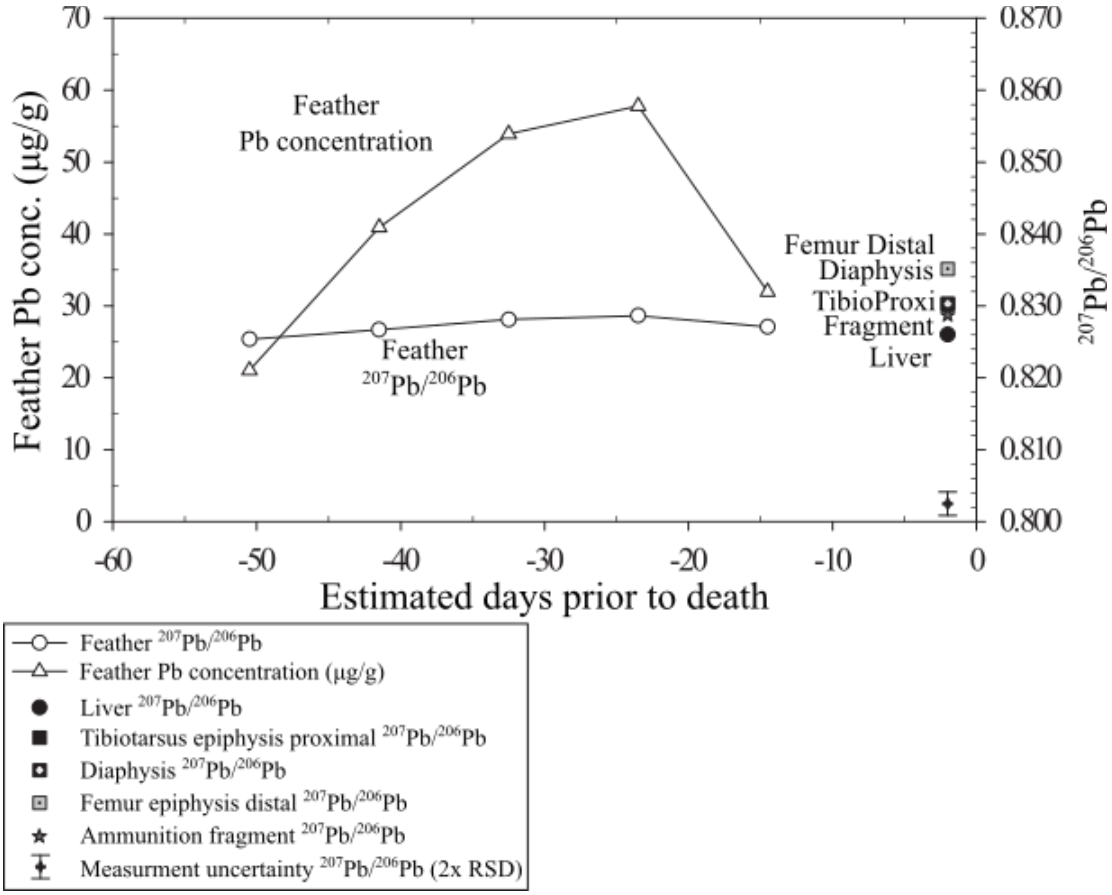

Figure 4.5 – Segments from condor 318 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and feather

was collected post-mortem. $^{207}Pb/^{206}Pb$ ratio for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square), femur epiphysis distal (grey square), and ammunition fragment (star) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure. For 318, Pb concentration in femur epiphysis distal was 45.3 µg/g, in tibiotarsus diaphysis was 23.4 µg/g, and in tibiotarsus epiphysis proximal was 81.3 µg/g. Liver Pb concentration was 15.8 µg/g.

As condor 318 was already exposed to Pb at the beginning of the timeframe captured by the growing feather analyzed, I estimated the pre-exposure conditions for it, i.e., before the exposure that is observed in the feather profile. I considered two different approaches for estimating the tissue $^{207}Pb/^{206}Pb$ ratio before the exposure event prior to death: (**318a**) $^{207}Pb/^{206}Pb$ ratio in the bones pre-exposure were the same as the $^{207}Pb/^{206}Pb$ ratio in the femur epiphysis distal post-exposure, 0.8351, (**318b**) bone $^{207}Pb/^{206}Pb$ ratio pre-exposure in 318 was the same as pre-exposure $^{207}Pb/^{206}Pb$ ratio found for condor 238, 0.8450. Condor 306 was not used because its pre-exposure $^{207}Pb/^{206}Pb$ ratio is close to the exposure source $^{207}Pb/^{206}Pb$ of condor 318. Scenario 318b is expected to be more realistic, since it is likely that femur epiphysis distal acquired Pb from the exposure source and would make the pre-exposure $^{207}Pb/^{206}Pb$ higher than the femur epiphysis distal $^{207}Pb/^{206}Pb$.

To estimate how long had passed since the exposure started, I assumed feather Pb concentration rises as an exponential curve in the few weeks following an exposure, based on the data from 238 (from 76 days until 21 days prior to death). I did an exponential regression with the curve generated by the feather Pb concentration profile and estimated that the exposure on condor 318 occurred 90 days prior to death based on the regression.

Lead uptake rates. Approximately 100% of Pb in liver for all three condors was attributed to the exposure event reflected in the feather, which corresponds to an uptake rate of 8%/week for condor 318, 11.3%/week for 238, and 12.3%/week for condor 306 (Table 4.4). In all the cases, liver had the highest Pb uptake rate among the tissues examined.

For condors 306 and 318, between 75% and 91% of the Pb in tibiotarsus epiphysis proximal (Table 4.4) was attributed to the exposure event, but for condor 238, only 17% of Pb in tibiotarsus epiphysis proximal was attributed to the exposure event. The difference is most likely due to the difference in the magnitude of Pb exposure, which is around 10-fold lower in 238 than 306 and 318. The uptake rate in tibiotarsus epiphysis proximal was around 9%/week for condors 306 and 318, but only 2%/week for condor 238 (Table 4.4).

In femur epiphysis distal, between 51% to 61% of Pb in bone was attributed to the exposure event, in condors 306 and 318 (Table 4.4), which yields a Pb uptake rate of 5%/week and 7%/week in femur epiphysis distal. In tibiotarsus diaphysis, between 2% and 8% of Pb were attributed to the event of exposure for condors 238 and 306 (Table 4.4), which yields a Pb uptake rate between 0.24%/week and 1%/week in tibiotarsus diaphysis. For condor 318, between 75% and 90% of the Pb in tibiotarsus diaphysis (Table 4.4) was attributed to the exposure event, which yields a Pb uptake rate of around 6%/week in tibiotarsus diaphysis.

Table 4.4 – Summary of data used that fit the criteria from Table 4.2 for mixing model analysis (Equation 1) to determine the Pb contribution into each bone based on the pre- and post-exposure $^{207}Pb/^{206}Pb$ ratios.

Studbook #	**Bone region**	**Pb concentration (µg/g)**	**Pre-exposure $^{207}Pb/^{206}Pb$ [i]**	**Exposure $^{207}Pb/^{206}Pb$ ratio [ii]**	**Post-exposure $^{207}Pb/^{206}Pb$ ratio [iii]**	**Duration of exposure estimated from feather (days) [iv]**	**Percentage contribution from exposure[v]**	**Estimated Pb uptake rate from exposure[vi]**
238	Tibiotarsus epiphysis proximal	10.6	0.8450	0.8028	0.8378	58	17%	2.0%/week
238	Femur epiphysis distal	5.11	0.8450	0.8028	0.8462	58	0%	0%/week
238	Tibiotarsus diaphysis	13.4	0.8450	0.8028	0.8441	58	2%	0.24%/week
238	Liver	1.99	0.8450	0.8028	0.8053	58	94%	11.3%/week
306	Tibiotarsus epiphysis proximal	164	0.8242	0.8154	0.8176	55	75%	9.4%/week
306	Femur epiphysis distal	61.1	0.8242	0.8154	0.8197	55	51%	6.4%/week
306	Tibiotarsus diaphysis	20.9	0.8242	0.8154	0.8235	55	8%	1.0%/week
306	Liver	207	0.8242	0.8154	0.8156	55	98%	12.3%/week
318a	Tibiotarsus epiphysis proximal	81.3	0.8351	0.8287	0.8302	90	77%	5.9%/week

318a	Tibiotarsus diaphysis	23.4	0.8351	0.8287 (fragment)	0.8303	90	75%	5.8%/week
318a	Liver	15.8	0.8351	0.8287	0.826	90	100%	7.7%/week
318b	Tibiotarsus epiphysis proximal	81.3	0.8450	0.8287	0.8302	90	91%	7.0%/week
318b	Femur epiphysis distal	45.3	0.8450	0.8287	0.8351	90	61%	4.7%/week
318b	Tibiotarsus diaphysis	23.4	0.8450	0.8287	0.8303	90	90%	6.9%/week
318b	Liver	15.8	0.8450	0.8287	0.826	90	100%	7.7%/week

[i] Pre-exposure $^{207}Pb/^{206}Pb$ was determined from segmented feather analysis, using the $^{207}Pb/^{206}Pb$ in a segment before the exposure event as determined by a change in feather $^{207}Pb/^{206}Pb$.

[ii] Exposure $^{207}Pb/^{206}Pb$ was determined from ammunition, when available, or from the feather segment with highest Pb concentration.

[iii] Post-exposure $^{207}Pb/^{206}Pb$ was measured for each bone region and liver.

[iv] Duration was estimated based on the number of days between the exposure event and death.

[v] Percentage contribution from the current exposure was calculated using Equation 1.

[vi] Pb uptake rate estimated in each tissue using percent contribution from the exposure and the estimated duration of exposure.

4.3.4 Pb concentration and $^{207}Pb/^{206}Pb$ ratio in feather, bone and liver for cases that did not fit criteria (Table 4.2).

For condors 445 and 502, that were likely not exposed to elevated amounts of Pb, bone Pb concentration was 0.210 µg/g on average and ranged from 0.080 to 0.400 µg/g (Appendix B, Figure S.1-S.2 and Table S.3). The $^{207}Pb/^{206}Pb$ ratio in bones was between 0.8227 and 0.8338, and liver Pb concentration was <1 µg/g ($^{207}Pb/^{206}Pb$ ratio ~0.8350). Feather Pb concentration ranged from 0.008 and 0.191 µg/g. The feather $^{207}Pb/^{206}Pb$ ratio for condors 445 and 502 ranged from 0.8262 to 0.8416.

Condors 245 and 345 had two events of Pb exposure over the timeframe of feather growth. For condor 245, the events had peak feather Pb concentration ~15 days and ~90 days prior to death (feather $^{207}Pb/^{206}Pb$ ratio of 0.8539 and 0.8655 and Pb concentration of ~3.8 and ~29 µg/g respectively, Appendix B, Figure S.4 and Table S.3). For condor 345, the events had peak feather Pb concentration ~15 and ~70 days prior to death ($^{207}Pb/^{206}Pb$ ratio of 0.8217 and 0.8265 and Pb concentration of ~23 and ~72 µg/g, respectively, Appendix B, Figure S.6 and Table S.3). Cases with two Pb exposure event did not fit the criteria for this study because it is not possible to determine the relative contribution of the two exposure events.

Condors 112, 286, and 458 had liver $^{207}Pb/^{206}Pb$ ratio and bone $^{207}Pb/^{206}Pb$ ratio values that were not measurably different. Differences in $^{207}Pb/^{206}Pb$ ratio are necessary to apply the mixing model (Equation 1). Condor 112 had an event of exposure greater than 60 days prior to death with feather Pb concentration that peaked ~60 days prior to death ($^{207}Pb/^{206}Pb$ ratio of 0.8086 and feather Pb concentration of ~39 µg/g), bone

$^{207}Pb/^{206}Pb$ ratio between 0.8131 and 0.8150, and liver $^{207}Pb/^{206}Pb$ ratio of 0.8134 (Appendix B, Figure S.3 and Table S.3). Condor 286 had an event of exposure peaking ~70 days prior to death ($^{207}Pb/^{206}Pb$ ratio of 0.8187 and feather Pb concentration of ~39 µg/g), bone $^{207}Pb/^{206}Pb$ ratio between 0.8170 and 0.8179, and liver $^{207}Pb/^{206}Pb$ ratio of 0.8190 (Appendix B, Figure S.5 and Table S.3). Condor 458 had an event of exposure peaking ~50 days prior to death with feather $^{207}Pb/^{206}Pb$ ratio of 0.8209 and feather Pb concentration of ~20 µg/g, bone $^{207}Pb/^{206}Pb$ ratio between 0.8205 and 0.8209, and liver $^{207}Pb/^{206}Pb$ ratio of 0.8206 (Appendix B, Figure S.7 and Table S.3).

4.4 Discussion

Lead isotopic composition ($^{207}Pb/^{206}Pb$ ratio) has been used to estimate the relative contribution of Pb from different sources into blood (Inskip *et al.*, 1996; Smith, Osterloh and Flegal, 1996; Gwiazda, Campbell and Smith, 2005). In this work, I used $^{207}Pb/^{206}Pb$ ratio to estimate the relative contribution of Pb from a specific exposure event into tibiotarsus epiphysis proximal, tibiotarsus diaphysis, femur epiphysis distal and liver. Then, I used the timeframes of Pb exposure, as estimated from segmented feather analysis (Church *et al.*, 2006; Finkelstein *et al.*, 2010, 2014) to assess the Pb incorporation rate into bone. In this study, 10 condors were analyzed, and three condors fit the criterion of one exposure event throughout the feather growth and differences in $^{207}Pb/^{206}Pb$ ratio between liver and other tissues. Based on the three condors used for the mixing model, Pb uptake rate varied by 10-fold between tibiotarsus epiphysis proximal and diaphysis. However, due to the limited number of samples, the specific values of uptake rates may require further confirmation.

Tibiotarsus epiphysis proximal had a Pb uptake 10-fold faster than tibiotarsus diaphysis for condors 238 and 306 (around 9%/week for tibiotarsus epiphysis proximal and between 0.3% and 1%/week for tibiotarsus diaphysis). Femur epiphysis distal had a Pb uptake around 6-fold faster than tibiotarsus diaphysis (around 6%/week for femur epiphysis distal and between 0.3% and 1%/week for tibiotarsus diaphysis). Epiphyses in both femur and tibiotarsus are composed of largely trabecular bone whereas diaphysis is composed of largely compact bone. There are no studies that evaluate the mineral or Pb turnover in large avian bones, but in White Leghorn hens bone mineral turnover rates of compact bone is ~0.7% and of trabecular bone is ~4% per month (Hurwitz, 1965). The 5.7-fold difference found in the turnover between compact and trabecular bone types in White Leghorns seems to agree with the findings in this study for Pb uptake in the epiphyses of femur and tibiotarsus and tibiotarsus diaphysis. In primates, the trabecular portion of tibia incorporated Pb 2.5-fold faster than compact portion of tibia, which are lower than the relative Pb uptake rates between trabecular and compact portions of tibiotarsus found in this study (Inskip *et al.*, 1996). Although the relative Pb uptake rates between trabecular and compact portions of tibiotarsus found in this study were higher, they may be influenced by the relative compact to trabecular composition of each bone region. The difference in Pb uptake between tibiotarsus diaphysis and tibiotarsus epiphysis proximal supports previous studies in eagles and swans where bone Pb was higher in epiphysis than diaphysis in birds that were acutely exposed to Pb (Ishii *et al.*, 2018).

When comparing the two epiphyses, tibiotarsus epiphysis proximal had an incorporation rate of 7% to 9%/week which is 1.4 to 1.6-fold higher than femur epiphysis distal. There are a couple of factors that may contribute to the difference in Pb uptake rate: differences in the relative amounts of trabecular and compact bone in tibiotarsus epiphysis proximal and femur diaphysis and difference in pneumatization between the bones. In condors, tibiotarsus is a non-pneumatized bone, whereas femur is pneumatized, which may reduce blood perfusion. Blood perfusion into bone is related to bone Pb uptake (O'Flaherty, 1993). Studies with eagles and swans have also reported bone Pb accumulation differs based on whether bone contains marrow or not (Ishii *et al.*, 2018).

The estimated Pb incorporation rates for tibiotarsus diaphysis and tibiotarsus epiphysis proximal were similar in condor 318, which is likely due to the uncertainty in the estimations of pre-exposure $^{207}Pb/^{206}Pb$ ratio and indicate an overestimate of the incorporation rate in tibiotarsus diaphysis. Since tibiotarsus diaphysis has a lower turnover rate (Hurwitz, 1965; Clarke, 2008), Pb residency time in tibiotarsus diaphysis is longer, and most likely reflects Pb from different exposures events. Therefore $^{207}Pb/^{206}Pb$ ratios would be expected to be a combination of the ratios from all the sources of prior exposure events, which could interfere with the estimates found. In humans, the half-life of Pb in trabecular bone is known to be faster than that of compact bone (Schütz *et al.*, 1987), Pb half-life in diaphysis has been reported to be between 5-10 years, whereas in trabecular bone it has been found to be of around 1 year (Hu, Rabinowitz and Smith, 1998). In red grouses, it was not possible to distinguish between

multiple events of exposure when sources of Pb had similar $^{207}Pb/^{206}Pb$ ratio (Thomas, Scheuhammer and Bond, 2009). Moreover, it is hard to determine how much of the current skeletal Pb burden was derived from past compared to more recent exposures (Smith, Osterloh and Flegal, 1996).

Lead incorporation into bones depends on the magnitude of Pb exposure. The results for condor 306 indicated a higher incorporation rate relative to condor 238. However, this is most likely attributable to the differences in the magnitude of Pb exposure. While condor 238 had an estimated maximum of ~114 µg/dL, condor 306 had a maximum of 1240 µg/dL blood Pb concentration, which is over 10-fold higher. For tibiotarsus epiphysis proximal and femur epiphysis distal, the incorporation rate for condors 306 and 318 was similar, which supports the argument that the incorporation rate is dependent on the magnitude of Pb exposure, since condor 318 had a peak of blood Pb estimated at 1100 µg/dL. Toxicokinetic models of Pb exposure consider a linear dependency between Pb incorporated into bone and blood Pb concentration (O'Flaherty, 1993; Fleming *et al.*, 1999). Therefore, the increased magnitude of Pb exposure for condors 306 and 318 is expected to increase the contribution of Pb in bone attributed to the exposure event.

For condor 318, there is higher uncertainty in the timeframe of exposure, since it is not clear from the feather profile, since the condor presented high feather Pb in the oldest feather segment. Moreover, the pre-exposure $^{207}Pb/^{206}Pb$ ratio for this condor cannot be accurately determined and the estimates made for this condor should be considered recognizing its uncertainty. Nevertheless, the exposure $^{207}Pb/^{206}Pb$ ratio is

accurately measured since the exposure source (ammunition fragment) was recovered from the GI tract of the condor carcass. The results for femur epiphysis distal seem counterintuitive, since it seems to have incorporated less Pb than tibiotarsus diaphysis. The most likely explanation to the higher incorporation in tibiotarsus diaphysis is that the $^{207}Pb/^{206}Pb$ of tibiotarsus diaphysis before was greatly influenced by past exposures to Pb, and so had a lower $^{207}Pb/^{206}Pb$ than the other bones before the exposure started.

The incorporation rate for Pb in the liver of condors 238, 306, and 318 showed that around 100% of Pb originated from the current exposure, which indicates that blood and liver Pb are very similar and that Pb uptake into liver is faster than the bone regions tested. In the cases of condor 306 and 318, the incorporation in liver and in tibiotarsus epiphysis proximal was similar (10 – 30% difference), suggesting that Pb in liver and tibiotarsus epiphysis proximal are both good surrogates of blood Pb.

The differences in uptake rate reported here can help explain the differences in Pb concentration found between different tibiotarsus epiphysis proximal and tibiotarsus diaphysis in condors that died of Pb poisoning in the previous chapters. In Chapter 3, condors that died of Pb poisoning, and thus, were acutely exposed to Pb, had Pb concentration in tibiotarsus epiphysis proximal 1.7-fold higher than femur epiphysis distal and 3-fold higher than tibiotarsus diaphysis. Whereas, condors that died of other causes had Pb concentration in tibiotarsus epiphysis proximal 0.8-fold lower than in femur epiphysis distal and only 1.6-fold higher than tibiotarsus diaphysis. The higher Pb uptake rates in tibiotarsus epiphysis proximal support that condors that acutely exposed to Pb have a higher Pb concentration in tibiotarsus epiphysis proximal.

4.5 Conclusion

In this chapter, I used $^{207}Pb/^{206}Pb$ ratio to inform about Pb toxicokinetics in bone, relying on segmented feather analysis to assess Pb exposure over the timeframe of feather growth in the months preceding the bird's death. I found that Pb uptake into bone depends on bone region. Tibiotarsus epiphysis proximal incorporated 10-fold more Pb than tibiotarsus diaphysis and 1.5-fold more Pb than femur epiphysis proximal. The difference in Pb uptake rate supports the findings from previous chapters that tibiotarsus epiphysis proximal had the highest Pb concentration in birds that were acutely exposed to Pb due to fastest Pb uptake among the three bone regions tested.

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Chapter 5) Conclusion

California Condors are critically endangered, and Pb is the primary cause of mortality and has impacts in condor health. Biomarkers for Pb exposure at sub-lethal levels are not well stablished in birds and the identification of a cause of death relies on the availability of fresh tissues, which is not always possible for necropsy.

Although bone Pb concentration has been recognized for many years as a biomarker for Pb exposure, it is unclear how Pb accumulates in various avian bones and bone regions. In this thesis I showed that Pb concentrations vary within the condor skeleton. I also observed that differences in bone Pb concentration in different bones are not simply an artifact of differences in bone mineral content. Regions that have faster turnover rates, such as largely trabecular bones were more sensitive to Pb uptake. As a consequence, the magnitude of Pb exposure, as well as informing whether a condor was Pb poisoned at time of death, inferred from bone differed substantially depending on which bone and/or bone region was analyzed.

Bone Pb concentrations, particularly in tibiotarsus epiphysis proximal and tibiotarsus diaphysis, can be used as an additional tool to help inform whether a condor was Pb poisoned in cases where a cause of death was not determined, in combination with feather Pb. Discriminant analyses using bone Pb concentration in tibiotarsus epiphysis proximal, tibiotarsus diaphysis, and femur epiphysis distal, were able to correctly classify over 80% of condors in regards to their Pb poisoning status. Bone Pb is a promising tool to help to improve the diagnose of Pb poisoning post-mortem in codors. I also found that age, number of free fly days, as well as integral and peak of

blood Pb over the lifetime of a condor were associated with cumulative Pb exposure, as reflected in bone Pb levels.

Since different bone types have different mineral turnover rates, and turnover rates also vary depending on the bone function, it is therefore reasonable to assume that Pb accumulation in different bones and bone types is not the same. Thus, in my final chapter, I investigated bone Pb uptake in femur epiphysis distal, tibiotarsus diaphysis and tibiotarsus epiphysis proximal of condors. Tibiotarsus epiphysis proximal had the highest Pb uptake rate among the bones tested, followed by femur epiphysis distal. Tibiotarsus diaphysis had the slowest Pb uptake rate.

My work can impact California Condor management by providing new biomarkers based on bone Pb concentration to inform a condor's poisoning status and help assess cumulative Pb exposure. The existence of better biomarkers for long term cumulative Pb exposure is a step towards better understanding of the relationship between Pb exposure in wild birds and negative health outcomes, such as reduction in bone mineral density and increased risk of fractures. The findings from this study could be used more broadly to better understand Pb exposure in other large avian species.

Appendix A: Complete data set for Chapters 2 and 3

Table S.1 – Summary of data used in Chapters 2 and 3. Bone Pb, Ca, and P concentration for each triplicate bone sample for each condor and bone region used.

Studbook #	BoneRegion	Replicate	Bone Pb ug/g	Final Ca bone mg/g	Final P bone mg/g
32	Femur epiphysis distal	A	1.85	220	97.3
32	Femur epiphysis distal	B	1.33	225	98.2
32	Femur epiphysis distal	C	1.62	209	91.3
32	Tibiotarsus epiphysis proximal	A	1.58	164	73.9
32	Tibiotarsus epiphysis proximal	B	1.68	189	83.3
32	Tibiotarsus epiphysis proximal	C	1.49	156	70.1
32	Tibiotarsus diaphysis	A	1.16	239	105
32	Tibiotarsus diaphysis	B	1.35	248	107
32	Tibiotarsus diaphysis	C	1.16	262	114
63	Femur epiphysis distal	A	3.82	237	104
63	Femur epiphysis distal	B	5.04	223	97.7
63	Femur epiphysis distal	C	4.08	233	102
63	Tibiotarsus epiphysis proximal	A	3.40	194	88.9
63	Tibiotarsus epiphysis proximal	B	2.59	164	75.2
63	Tibiotarsus epiphysis proximal	C	3.38	196	89.4
63	Tibiotarsus diaphysis	A	3.00	268	118
63	Tibiotarsus diaphysis	B	2.64	260	115
63	Tibiotarsus diaphysis	C	2.27	258	114
102	Tibiotarsus epiphysis proximal	A	44.2	139	64.7
102	Tibiotarsus epiphysis proximal	B	41.6	140	65.6
102	Tibiotarsus epiphysis proximal	C	13.4	52	24.7
102	Tibiotarsus diaphysis	A	17.4	252	114
102	Tibiotarsus diaphysis	B	18.6	259	117

102	Tibiotarsus diaphysis	C	14.1	245	110
112	Femur epiphysis distal	A	50.6	224	99.8
112	Femur epiphysis distal	B	47.5	210	93.2
112	Femur epiphysis distal	C	55.9	199	89.2
112	Tibiotarsus epiphysis proximal	A	52.5	204	92.8
112	Tibiotarsus epiphysis proximal	B	48.4	382	174
112	Tibiotarsus epiphysis proximal	C	59.5	192	87.3
112	Tibiotarsus diaphysis	A	26.3	216	97.7
112	Tibiotarsus diaphysis	B	26.6	241	109
112	Tibiotarsus diaphysis	C	29.3	252	114
125	Femur epiphysis distal	A	30.6	213	93.7
125	Femur epiphysis distal	B	31.4	206	91.4
125	Femur epiphysis distal	C	36.6	212	95.7
125	Tibiotarsus epiphysis proximal	A	14.8	138	70.1
125	Tibiotarsus epiphysis proximal	B	15.1	123	66.1
125	Tibiotarsus epiphysis proximal	C	18.7	114	52.1
125	Tibiotarsus diaphysis	A	23.3	241	106
125	Tibiotarsus diaphysis	B	19.8	240	106
125	Tibiotarsus diaphysis	C	19.7	232	101
192	Femur epiphysis distal	A	54.5	217	97.7
192	Femur epiphysis distal	B	70.3	208	92.6
192	Femur epiphysis distal	C	46.5	209	94.8
192	Tibiotarsus epiphysis proximal	A	97.0	208	94.9
192	Tibiotarsus epiphysis proximal	B	110	197	90.8
192	Tibiotarsus epiphysis proximal	C	90.4	187	84.7
192	Tibiotarsus diaphysis	A	35.5	230	104
192	Tibiotarsus diaphysis	B	52.0	233	106
192	Tibiotarsus diaphysis	C	46.3	241	108
195	Femur epiphysis distal	A	11.4	200	93.2
195	Femur epiphysis distal	B	10.7	229	106
195	Femur epiphysis distal	C	10.0	201	95.5

195	Tibiotarsus epiphysis proximal	A	10.7	146	68.1
195	Tibiotarsus epiphysis proximal	B	11.5	138	65.7
195	Tibiotarsus epiphysis proximal	C	14.5	197	92.1
195	Tibiotarsus diaphysis	A	8.31	260	116
195	Tibiotarsus diaphysis	B	3.97	127	57.6
195	Tibiotarsus diaphysis	C	6.88	266	119
238	Femur epiphysis distal	A	5.61	302	125
238	Femur epiphysis distal	B	4.28	128	58.4
238	Femur epiphysis distal	C	5.45	255	116
238	Tibiotarsus epiphysis proximal	A	7.10	100	47.6
238	Tibiotarsus epiphysis proximal	B	14.6	179	71.5
238	Tibiotarsus epiphysis proximal	C	10.1	123	57.0
238	Tibiotarsus diaphysis	A	9.78	197	89.8
238	Tibiotarsus diaphysis	B	13.9	213	96.7
238	Tibiotarsus diaphysis	C	16.4	186	83.4
242	Femur epiphysis distal	A	30.2	239	105
242	Femur epiphysis distal	B	30.6	313	138
242	Femur epiphysis distal	C	32.6	278	123
242	Tibiotarsus epiphysis proximal	A	76.9	195	86.8
242	Tibiotarsus epiphysis proximal	B	79.8	193	87.1
242	Tibiotarsus epiphysis proximal	C	71.1	190	87.7
242	Tibiotarsus diaphysis	A	25.6	268	120
242	Tibiotarsus diaphysis	B	18.1	246	110
242	Tibiotarsus diaphysis	C	20.9	256	113
245	Femur epiphysis distal	A	16.0	218	97.4
245	Femur epiphysis distal	B	16.3	213	94.9
245	Femur epiphysis distal	C	16.5	209	93.6
245	Tibiotarsus epiphysis proximal	A	19.0	184	84.4
245	Tibiotarsus epiphysis proximal	B	18.2	164	75.2

245	Tibiotarsus epiphysis proximal	C	20.7	156	73.0
245	Tibiotarsus diaphysis	A	7.82	214	98.9
245	Tibiotarsus diaphysis	B	12.2	249	111
245	Tibiotarsus diaphysis	C	10.2	197	91.6
246	Femur epiphysis distal	A	48.0	212	96.5
246	Femur epiphysis distal	B	64.5	210	95.6
246	Femur epiphysis distal	C	62.0	211	95.0
246	Tibiotarsus epiphysis proximal	A	61.5	188	87.4
246	Tibiotarsus epiphysis proximal	B	69.1	182	84.2
246	Tibiotarsus epiphysis proximal	C	59.2	194	90.0
246	Tibiotarsus diaphysis	A	31.6	245	111
246	Tibiotarsus diaphysis	B	29.0	246	111
246	Tibiotarsus diaphysis	C	28.3	246	110
265	Femur epiphysis distal	A	26.0	225	100
265	Femur epiphysis distal	B	23.1	224	101
265	Femur epiphysis distal	C	8.55	91	41.0
265	Tibiotarsus epiphysis proximal	A	43.4	173	81.0
265	Tibiotarsus epiphysis proximal	B	34.8	185	86.7
265	Tibiotarsus epiphysis proximal	C	31.8	160	75.3
265	Tibiotarsus diaphysis	A	19.1	273	123
265	Tibiotarsus diaphysis	B	20.8	274	125
265	Tibiotarsus diaphysis	C	21.4	272	123
272	Femur epiphysis distal	A	35.5	213	97.1
272	Femur epiphysis distal	B	34.8	215	96.5
272	Femur epiphysis distal	C	37.6	216	98.0
272	Tibiotarsus epiphysis proximal	A	48.0	149	70.0
272	Tibiotarsus epiphysis proximal	B	76.7	189	87.7
272	Tibiotarsus epiphysis proximal	C	86.3	117	58.2
272	Tibiotarsus diaphysis	A	18.9	248	114
272	Tibiotarsus diaphysis	B	22.3	253	113

272	Tibiotarsus diaphysis	C	22.9	259	115
286	Femur epiphysis distal	A	26.9	199	89.0
286	Femur epiphysis distal	B	25.7	200	89.3
286	Femur epiphysis distal	C	13.5	214	95.3
286	Tibiotarsus epiphysis proximal	A	13.3	203	93.3
286	Tibiotarsus epiphysis proximal	B	17.2	189	86.9
286	Tibiotarsus epiphysis proximal	C	17.9	197	90.9
286	Tibiotarsus diaphysis	A	8.63	258	116
286	Tibiotarsus diaphysis	B	9.22	271	121
286	Tibiotarsus diaphysis	C	9.24	257	114
299	Femur epiphysis distal	A	62.2	234	105
299	Femur epiphysis distal	B	103	204	91.8
299	Femur epiphysis distal	C	70.3	233	104
299	Tibiotarsus epiphysis proximal	A	100	200	91.8
299	Tibiotarsus epiphysis proximal	B	80.4	159	74.0
299	Tibiotarsus epiphysis proximal	C	83.1	163	75.3
299	Tibiotarsus diaphysis	A	42.5	242	108
299	Tibiotarsus diaphysis	B	49.4	254	114
299	Tibiotarsus diaphysis	C	36.0	256	115
301	Femur diaphysis	A	6.73	238	104
301	Femur diaphysis	B	6.03	232	105
301	Femur diaphysis	C	6.49	191	82.6
301	Femur epiphysis distal	A	16.8	173	75.5
301	Femur epiphysis distal	B	16.2	183	80.8
301	Femur epiphysis distal	C	16.6	190	84.1
301	Femur epiphysis proximal	A	14.0	214	97.5
301	Femur epiphysis proximal	B	9.93	218	96.2
301	Femur epiphysis proximal	C	8.69	202	90.8
301	Humerus diaphysis	A	6.15	236	107
301	Humerus diaphysis	B	5.41	226	100
301	Humerus diaphysis	C	6.25	226	99.1
301	Humerus epiphysis distal	A	7.28	209	92.6
301	Humerus epiphysis distal	B	7.58	219	96.3

301	Humerus epiphysis distal	C	10.7	223	98.3
301	Humerus epiphysis proximal	A	10.4	211	92.4
301	Humerus epiphysis proximal	B	13.1	217	98.2
301	Humerus epiphysis proximal	C	9.59	227	101
301	Tibiotarsus diaphysis	A	6.80	185	79.3
301	Tibiotarsus diaphysis	B	6.26	250	111
301	Tibiotarsus diaphysis	C	6.03	238	107
301	Tibiotarsus epiphysis distal	A	7.97	150	66.6
301	Tibiotarsus epiphysis distal	B	9.25	157	68.3
301	Tibiotarsus epiphysis distal	C	12.9	145	64.0
301	Tibiotarsus epiphysis proximal	A	13.0	156	69.3
301	Tibiotarsus epiphysis proximal	B	13.0	146	67.0
301	Tibiotarsus epiphysis proximal	C	11.2	174	79.4
306	Femur epiphysis distal	A	44.3	209	89.1
306	Femur epiphysis distal	B	43.6	220	93.9
306	Femur epiphysis distal	C	72.5	210	90.3
306	Tibiotarsus epiphysis proximal	A	140	179	78.3
306	Tibiotarsus epiphysis proximal	B	138	177	78.7
306	Tibiotarsus epiphysis proximal	C	206	169	76.2
306	Tibiotarsus diaphysis	A	16.5	243	106
306	Tibiotarsus diaphysis	B	13.1	244	106
306	Tibiotarsus diaphysis	C	15.1	216	92.5
307	Femur epiphysis distal	A	27.8	219	97.3
307	Femur epiphysis distal	B	26.7	219	98.7
307	Femur epiphysis distal	C	34.1	200	90.0
307	Tibiotarsus epiphysis proximal	A	35.9	185	84.0
307	Tibiotarsus epiphysis proximal	B	34.0	182	82.4
307	Tibiotarsus epiphysis proximal	C	35.0	197	88.3
307	Tibiotarsus diaphysis	A	14.7	250	112
307	Tibiotarsus diaphysis	B	17.7	258	116
307	Tibiotarsus diaphysis	C	14.8	250	113

312	Femur diaphysis	A	30.4	253	112
312	Femur diaphysis	B	25.7	243	108
312	Femur diaphysis	C	27.8	244	109
312	Femur epiphysis distal	A	103	210	94.3
312	Femur epiphysis distal	B	59.9	224	99.0
312	Femur epiphysis distal	C	69.3	211	94.5
312	Femur epiphysis proximal	A	49.4	202	91.4
312	Femur epiphysis proximal	B	47.3	226	100
312	Femur epiphysis proximal	C	50.9	236	108
312	Humerus diaphysis	A	23.5	264	118
312	Humerus diaphysis	B	24.0	252	112
312	Humerus diaphysis	C	25.2	256	114
312	Humerus epiphysis distal	A	32.8	231	103
312	Humerus epiphysis distal	B	33.2	235	105
312	Humerus epiphysis distal	C	50.6	239	106
312	Humerus epiphysis proximal	A	78.9	237	105
312	Humerus epiphysis proximal	B	67.6	240	106
312	Humerus epiphysis proximal	C	50.7	239	105
312	Tibiotarsus diaphysis	A	49.9	270	123
312	Tibiotarsus diaphysis	B	38.2	250	111
312	Tibiotarsus diaphysis	C	48.3	257	114
312	Tibiotarsus epiphysis distal	A	68.2	150	68.7
312	Tibiotarsus epiphysis distal	B	47.9	159	70.7
312	Tibiotarsus epiphysis distal	C	75.2	175	78.1
312	Tibiotarsus epiphysis proximal	A	220	166	81.2
312	Tibiotarsus epiphysis proximal	B	146	177	82.0
312	Tibiotarsus epiphysis proximal	C	170	175	80.5
318	Femur epiphysis distal	A	35.4	208	91.2
318	Femur epiphysis distal	B	42.8	192	85.7
318	Femur epiphysis distal	C	45.8	225	93.8
318	Tibiotarsus epiphysis proximal	A	117	147	67.4
318	Tibiotarsus epiphysis proximal	B	106	163	75.5
318	Tibiotarsus epiphysis proximal	C	71.0	172	81.7

318	Tibiotarsus diaphysis	A	20.6	250	112
318	Tibiotarsus diaphysis	B	22.0	248	109
318	Tibiotarsus diaphysis	C	23.3	233	102
345	Femur diaphysis	A	34.7	240	107
345	Femur diaphysis	B	28.0	251	112
345	Femur diaphysis	C	30.5	252	111
345	Femur epiphysis distal	A	64.4	205	90.0
345	Femur epiphysis distal	B	57.8	197	88.6
345	Femur epiphysis distal	C	95.2	182	79.7
345	Femur epiphysis proximal	A	26.9	211	92.3
345	Femur epiphysis proximal	B	26.9	207	92.2
345	Femur epiphysis proximal	C	28.8	192	84.7
345	Humerus diaphysis	A	19.3	204	88.7
345	Humerus diaphysis	B	24.8	238	104
345	Humerus diaphysis	C	22.6	243	106
345	Humerus epiphysis distal	A	37.8	228	101
345	Humerus epiphysis distal	B	31.0	226	99.3
345	Humerus epiphysis distal	C	23.7	209	90.4
345	Humerus epiphysis proximal	A	37.9	183	79.6
345	Humerus epiphysis proximal	B	46.7	201	88.0
345	Humerus epiphysis proximal	C	40.6	234	102
345	Tibiotarsus diaphysis	A	28.5	234	104
345	Tibiotarsus diaphysis	B	27.4	228	100
345	Tibiotarsus diaphysis	C	25.0	228	101
345	Tibiotarsus epiphysis distal	A	49.0	203	92.7
345	Tibiotarsus epiphysis distal	B	47.3	181	78.8
345	Tibiotarsus epiphysis distal	C	39.7	180	80.8
345	Tibiotarsus epiphysis proximal	A	60.1	207	96.1
345	Tibiotarsus epiphysis proximal	B	61.5	144	66.3
345	Tibiotarsus epiphysis proximal	C	53.1	206	93.6
356	Femur epiphysis distal	A	1.31	218	96.7
356	Femur epiphysis distal	B	1.47	195	87.5
356	Femur epiphysis distal	C	1.24	213	96.7
356	Tibiotarsus epiphysis proximal	A	0.900	122	57.5

356	Tibiotarsus epiphysis proximal	B	0.715	124	60.0
356	Tibiotarsus epiphysis proximal	C	0.942	157	73.5
356	Tibiotarsus diaphysis	A	1.20	249	113
356	Tibiotarsus diaphysis	B	1.33	229	105
356	Tibiotarsus diaphysis	C	0.569	112	50.8
408	Femur epiphysis distal	A	14.4	184	84.6
408	Femur epiphysis distal	B	17.5	184	85.7
408	Femur epiphysis distal	C	14.2	227	101
408	Tibiotarsus epiphysis proximal	A	10.1	174	76.5
408	Tibiotarsus epiphysis proximal	B	11.3	165	75.2
408	Tibiotarsus epiphysis proximal	C	10.5	161	73.5
408	Tibiotarsus diaphysis	A	8.74	246	112
408	Tibiotarsus diaphysis	B	5.64	247	112
408	Tibiotarsus diaphysis	C	6.23	246	111
411	Femur epiphysis distal	A	94.4	196	88.2
411	Femur epiphysis distal	B	63.4	220	97.8
411	Femur epiphysis distal	C	86.0	225	99.6
411	Tibiotarsus epiphysis proximal	A	73.2	117	52.4
411	Tibiotarsus epiphysis proximal	B	99.6	139	62.5
411	Tibiotarsus epiphysis proximal	C	92.0	153	69.5
411	Tibiotarsus diaphysis	A	46.0	259	115
411	Tibiotarsus diaphysis	B	39.7	272	121
411	Tibiotarsus diaphysis	C	41.6	254	113
412	Femur diaphysis	A	19.4	82	36.5
412	Femur diaphysis	B	18.5	218	95.0
412	Femur diaphysis	C	15.2	245	110
412	Femur epiphysis distal	A	38.6	192	84.0
412	Femur epiphysis distal	B	35.4	206	88.7
412	Femur epiphysis distal	C	31.4	180	77.6
412	Femur epiphysis proximal	A	21.3	223	97.8
412	Femur epiphysis proximal	B	20.6	231	101

412	Femur epiphysis proximal	C	18.9	225	100
412	Humerus diaphysis	A	11.2	245	107
412	Humerus diaphysis	B	11.3	246	109
412	Humerus diaphysis	C	11.0	239	104
412	Humerus epiphysis distal	A	11.6	215	94.1
412	Humerus epiphysis distal	B	21.9	203	89.3
412	Humerus epiphysis distal	C	16.0	214	92.2
412	Humerus epiphysis proximal	A	17.0	220	94.7
412	Humerus epiphysis proximal	B	24.0	230	101
412	Humerus epiphysis proximal	C	23.7	228	100
412	Tibiotarsus diaphysis	A	13.2	242	108
412	Tibiotarsus diaphysis	B	13.7	239	106
412	Tibiotarsus diaphysis	C	11.9	239	102
412	Tibiotarsus epiphysis distal	A	15.2	177	75.9
412	Tibiotarsus epiphysis distal	B	18.4	175	75.2
412	Tibiotarsus epiphysis distal	C	15.5	203	88.8
412	Tibiotarsus epiphysis proximal	A	19.2	180	75.3
412	Tibiotarsus epiphysis proximal	B	20.7	196	87.5
412	Tibiotarsus epiphysis proximal	C	18.2	189	82.8
445	Femur diaphysis	A	0.081	220	97.7
445	Femur diaphysis	B	0.097	224	100
445	Femur diaphysis	C	0.092	234	104
445	Femur epiphysis distal	A	0.108	184	76.1
445	Femur epiphysis distal	B	0.104	191	84.9
445	Femur epiphysis distal	C	0.088	197	88.5
445	Femur epiphysis proximal	A	0.067	199	91.8
445	Femur epiphysis proximal	B	0.073	200	92.7
445	Femur epiphysis proximal	C	0.075	201	92.2
445	Humerus diaphysis	A	0.063	226	101
445	Humerus diaphysis	B	0.064	297	109
445	Humerus diaphysis	C	0.080	238	108
445	Humerus epiphysis distal	A	0.059	274	100
445	Humerus epiphysis distal	B	0.079	216	96.7
445	Humerus epiphysis distal	C	0.063	211	95.3
445	Humerus epiphysis proximal	A	0.063	219	97.4

445	Humerus epiphysis proximal	B	0.061	219	100
445	Humerus epiphysis proximal	C	0.088	219	98.2
445	Tibiotarsus diaphysis	A	0.076	210	94.3
445	Tibiotarsus diaphysis	B	0.088	219	97.7
445	Tibiotarsus diaphysis	C	0.083	179	81.0
445	Tibiotarsus epiphysis distal	A	0.083	158	71.8
445	Tibiotarsus epiphysis distal	B	0.149	156	70.5
445	Tibiotarsus epiphysis distal	C	0.185	126	57.1
445	Tibiotarsus epiphysis proximal	A	0.115	189	89.2
445	Tibiotarsus epiphysis proximal	B	0.121	183	83.8
445	Tibiotarsus epiphysis proximal	C	0.126	209	95.1
458	Femur diaphysis	A	7.18	241	111
458	Femur diaphysis	B	8.13	246	113
458	Femur diaphysis	C	8.63	257	115
458	Femur epiphysis distal	A	14.1	225	97.5
458	Femur epiphysis distal	B	11.0	235	103
458	Femur epiphysis distal	C	13.1	230	104
458	Femur epiphysis proximal	A	11.2	246	111
458	Femur epiphysis proximal	B	9.58	237	105
458	Femur epiphysis proximal	C	10.8	255	113
458	Humerus diaphysis	A	6.02	250	111
458	Humerus diaphysis	B	5.81	251	110
458	Humerus diaphysis	C	5.76	245	112
458	Humerus epiphysis distal	A	8.01	234	106
458	Humerus epiphysis distal	B	11.6	234	104
458	Humerus epiphysis distal	C	6.31	223	100
458	Humerus epiphysis proximal	A	12.0	236	106
458	Humerus epiphysis proximal	B	15.4	238	107
458	Humerus epiphysis proximal	C	12.2	224	101
458	Tibiotarsus diaphysis	A	8.04	244	110
458	Tibiotarsus diaphysis	B	8.70	249	114
458	Tibiotarsus diaphysis	C	4.72	218	98.8
458	Tibiotarsus epiphysis distal	A	16.7	228	103
458	Tibiotarsus epiphysis distal	B	15.2	228	104
458	Tibiotarsus epiphysis distal	C	8.69	187	83.7

458	Tibiotarsus epiphysis proximal	A	10.4	204	93.2
458	Tibiotarsus epiphysis proximal	B	12.8	168	74.2
458	Tibiotarsus epiphysis proximal	C	15.4	191	86.4
478	Femur diaphysis	A	17.2	273	121
478	Femur diaphysis	B	14.4	264	114
478	Femur diaphysis	C	12.9	263	120
478	Femur epiphysis distal	A	27.9	218	102
478	Femur epiphysis distal	B	25.9	143	67.2
478	Femur epiphysis distal	C	22.4	219	102
478	Femur epiphysis proximal	A	15.0	262	119
478	Femur epiphysis proximal	B	13.0	261	121
478	Femur epiphysis proximal	C	16.2	306	117
478	Humerus diaphysis	A	12.5	221	102
478	Humerus diaphysis	B	10.8	220	102
478	Humerus diaphysis	C	11.2	225	102
478	Humerus epiphysis distal	A	24.8	185	85.6
478	Humerus epiphysis distal	B	24.6	199	91.8
478	Humerus epiphysis distal	C	14.3	192	87.8
478	Humerus epiphysis proximal	A	18.3	247	104
478	Humerus epiphysis proximal	B	17.4	206	88.4
478	Humerus epiphysis proximal	C	18.9	209	94.8
478	Tibiotarsus diaphysis	A	12.1	218	98.9
478	Tibiotarsus diaphysis	B	13.5	216	96.8
478	Tibiotarsus diaphysis	C	10.7	236	102
478	Tibiotarsus epiphysis distal	A	32.1	177	80.0
478	Tibiotarsus epiphysis distal	B	29.9	178	83.3
478	Tibiotarsus epiphysis distal	C	27.0	176	82.0
478	Tibiotarsus epiphysis proximal	A	44.4	177	82.1
478	Tibiotarsus epiphysis proximal	B	45.4	192	89.1
478	Tibiotarsus epiphysis proximal	C	22.9	180	85.5
499	Femur epiphysis distal	A	9.80	231	105
499	Femur epiphysis distal	B	9.90	213	96.8
499	Femur epiphysis distal	C	6.21	226	103

499	Tibiotarsus epiphysis proximal	A	7.59	148	70.7
499	Tibiotarsus epiphysis proximal	B	9.88	150	72.0
499	Tibiotarsus epiphysis proximal	C	9.02	147	70.0
499	Tibiotarsus diaphysis	A	6.34	264	120
499	Tibiotarsus diaphysis	B	5.81	258	118
499	Tibiotarsus diaphysis	C	5.89	262	119
502	Femur diaphysis	A	0.277	243	108
502	Femur diaphysis	B	0.228	256	113
502	Femur diaphysis	C	0.265	252	113
502	Femur epiphysis distal	A	0.435	222	97.5
502	Femur epiphysis distal	B	0.341	239	108
502	Femur epiphysis distal	C	0.436	229	102
502	Femur epiphysis proximal	A	0.332	241	106
502	Femur epiphysis proximal	B	0.335	219	95.6
502	Femur epiphysis proximal	C	0.301	230	101
502	Humerus diaphysis	A	0.200	248	109
502	Humerus diaphysis	B	0.217	246	108
502	Humerus diaphysis	C	0.208	243	107
502	Humerus epiphysis distal	A	0.284	237	104
502	Humerus epiphysis distal	B	0.261	228	102
502	Humerus epiphysis distal	C	0.293	235	104
502	Humerus epiphysis proximal	A	0.350	170	76.0
502	Humerus epiphysis proximal	B	0.352	237	106
502	Humerus epiphysis proximal	C	0.310	231	102
502	Tibiotarsus diaphysis	A	0.218	261	115
502	Tibiotarsus diaphysis	B	0.244	256	112
502	Tibiotarsus diaphysis	C	0.219	261	114
502	Tibiotarsus epiphysis distal	A	0.317	188	81.6
502	Tibiotarsus epiphysis distal	B	0.392	170	74.9
502	Tibiotarsus epiphysis distal	C	0.378	172	78.4
502	Tibiotarsus epiphysis proximal	A	0.353	162	70.8
502	Tibiotarsus epiphysis proximal	B	0.321	144	65.3
502	Tibiotarsus epiphysis proximal	C	0.344	170	72.8

511	Femur epiphysis distal	A	0.512	221	99.6
511	Femur epiphysis distal	B	0.426	234	105
511	Femur epiphysis distal	C	0.432	223	100
511	Tibiotarsus epiphysis proximal	A	0.764	201	91.4
511	Tibiotarsus epiphysis proximal	B	0.583	208	95.8
511	Tibiotarsus epiphysis proximal	C	0.560	207	94.1
511	Tibiotarsus diaphysis	A	0.573	241	109
511	Tibiotarsus diaphysis	B	0.422	212	96.9
511	Tibiotarsus diaphysis	C	0.509	245	110
512	Femur epiphysis distal	A	2.80	203	91.7
512	Femur epiphysis distal	B	3.24	214	96.2
512	Femur epiphysis distal	C	3.85	205	93.7
512	Tibiotarsus epiphysis proximal	A	1.92	168	78.3
512	Tibiotarsus epiphysis proximal	B	1.99	148	68.6
512	Tibiotarsus epiphysis proximal	C	1.67	145	67.4
512	Tibiotarsus diaphysis	A	1.22	260	116
512	Tibiotarsus diaphysis	B	1.14	250	112
512	Tibiotarsus diaphysis	C	1.38	254	114
536	Femur epiphysis distal	A	4.47	235	107
536	Femur epiphysis distal	B	5.69	215	97.9
536	Femur epiphysis distal	C	5.33	229	104
536	Tibiotarsus epiphysis proximal	A	8.55	168	77.0
536	Tibiotarsus epiphysis proximal	B	9.49	171	78.7
536	Tibiotarsus epiphysis proximal	C	9.07	189	87.1
536	Tibiotarsus diaphysis	A	3.43	245	112
536	Tibiotarsus diaphysis	B	3.93	261	120
536	Tibiotarsus diaphysis	C	4.63	268	123
553	Femur diaphysis	A	18.0	227	103
553	Femur diaphysis	B	15.8	228	104
553	Femur diaphysis	C	16.9	239	109
553	Femur epiphysis distal	A	19.4	219	99.6

553	Femur epiphysis distal	B	17.5	207	93.9
553	Femur epiphysis distal	C	14.7	215	96.3
553	Femur epiphysis proximal	A	26.3	205	94.5
553	Femur epiphysis proximal	B	23.5	223	103
553	Femur epiphysis proximal	C	29.8	210	93.7
553	Femur metaphysis proximal	A	33.2	215	94.9
553	Femur metaphysis proximal	B	14.1	238	106
553	Femur metaphysis proximal	C	20.8	209	90.0
553	Fibula	A	48.6	241	112
553	Fibula	B	44.3	236	112
553	Fibula	C	42.2	239	112
553	Humerus diaphysis	A	11.2	221	101
553	Humerus diaphysis	B	11.9	248	112
553	Humerus diaphysis	C	11.5	244	111
553	Humerus epiphysis distal	A	40.5	197	90.9
553	Humerus epiphysis distal	B	34.9	207	93.4
553	Humerus epiphysis distal	C	37.5	209	96.3
553	Humerus epiphysis proximal	A	23.9	232	107
553	Humerus epiphysis proximal	B	32.4	212	98.3
553	Humerus epiphysis proximal	C	29.0	227	103
553	Humerus metaphysis proximal	A	14.7	190	82.2
553	Humerus metaphysis proximal	B	20.0	215	94.3
553	Humerus metaphysis proximal	C	23.6	233	112
553	Sternum base	A	33.6	199	88.8
553	Sternum base	B	29.2	189	91.1
553	Sternum base	C	13.9	203	99.1
553	Sternum keel	A	52.7	198	89.5
553	Tibiotarsus diaphysis	A	22.7	212	96.5
553	Tibiotarsus diaphysis	B	22.7	220	97.0
553	Tibiotarsus diaphysis	C	21.2	247	114
553	Tibiotarsus epiphysis distal	A	20.0	201	90.6
553	Tibiotarsus epiphysis distal	B	15.7	218	101
553	Tibiotarsus epiphysis distal	C	26.0	194	86.5
553	Tibiotarsus epiphysis proximal	A	122	154	68.2
553	Tibiotarsus epiphysis proximal	B	124	185	86.5

553	Tibiotarsus epiphysis proximal	C	127	172	83.9
553	Tibiotarsus metaphysis proximal	A	60.4	224	106
553	Tibiotarsus metaphysis proximal	B	49.2	206	98.0
553	Tibiotarsus metaphysis proximal	C	56.6	196	88.2
553	Vertebra 10th	A	61.8	153	71.2
553	Vertebra 17th	A	54.6	185	87.2
553	Vertebra 17th	B	26.9	172	82.2
553	Vertebra 17th	C	32.4	165	79.1
553	Vertebra 3rd	A	37.1	199	89.3
598	Femur epiphysis distal	A	42.8	232	106
598	Femur epiphysis distal	B	35.7	220	99.1
598	Femur epiphysis distal	C	40.0	216	97.6
598	Tibiotarsus epiphysis proximal	A	31.1	166	76.4
598	Tibiotarsus epiphysis proximal	B	30.4	156	73.2
598	Tibiotarsus epiphysis proximal	C	28.3	178	82.4
598	Tibiotarsus diaphysis	A	25.2	261	117
598	Tibiotarsus diaphysis	B	24.1	251	113
598	Tibiotarsus diaphysis	C	23.4	252	113
615	Femur epiphysis distal	A	16.3	194	89.1
615	Femur epiphysis distal	B	17.1	210	95.4
615	Femur epiphysis distal	C	15.5	173	80.9
615	Tibiotarsus epiphysis proximal	A	25.2	152	70.3
615	Tibiotarsus epiphysis proximal	B	24.1	145	67.4
615	Tibiotarsus epiphysis proximal	C	25.7	167	77.0
615	Tibiotarsus diaphysis	A	14.1	238	108
615	Tibiotarsus diaphysis	B	11.7	246	111
615	Tibiotarsus diaphysis	C	11.2	200	95.1
630	Femur diaphysis	A	4.82	231	106
630	Femur diaphysis	B	5.52	230	105
630	Femur diaphysis	C	4.08	195	88.4

630	Femur epiphysis distal	A	5.99	202	91.7
630	Femur epiphysis distal	B	7.61	190	87.0
630	Femur epiphysis distal	C	6.15	204	92.7
630	Femur epiphysis proximal	A	5.49	205	93.4
630	Femur epiphysis proximal	B	5.93	204	94.4
630	Femur epiphysis proximal	C	5.11	201	90.4
630	Humerus diaphysis	A	4.32	236	104
630	Humerus diaphysis	B	4.26	237	106
630	Humerus diaphysis	C	4.02	224	102
630	Humerus epiphysis distal	A	6.28	213	94.2
630	Humerus epiphysis distal	B	4.38	225	102
630	Humerus epiphysis distal	C	4.60	211	94.1
630	Humerus epiphysis proximal	A	6.31	200	90.5
630	Humerus epiphysis proximal	B	7.28	211	96.6
630	Humerus epiphysis proximal	C	6.87	185	85.9
630	Tibiotarsus diaphysis	A	6.01	224	103
630	Tibiotarsus diaphysis	B	5.52	210	95.5
630	Tibiotarsus diaphysis	C	6.48	237	109
630	Tibiotarsus epiphysis distal	A	6.56	146	66.6
630	Tibiotarsus epiphysis distal	B	5.96	166	77.5
630	Tibiotarsus epiphysis distal	C	7.44	160	72.1
630	Tibiotarsus epiphysis proximal	A	7.78	199	91.6
630	Tibiotarsus epiphysis proximal	B	7.54	186	88.2
630	Tibiotarsus epiphysis proximal	C	7.53	158	73.4
639	Femur diaphysis	A	0.784	239	106
639	Femur diaphysis	B	0.750	236	105
639	Femur diaphysis	C	0.771	237	106
639	Femur epiphysis distal	A	0.891	201	87.6
639	Femur epiphysis distal	B	0.976	204	89.5
639	Femur epiphysis distal	C	0.934	198	87.9
639	Femur epiphysis proximal	A	0.751	205	91.4
639	Femur epiphysis proximal	B	0.816	206	90.1
639	Femur epiphysis proximal	C	0.841	174	78.3
639	Humerus diaphysis	A	0.791	241	106
639	Humerus diaphysis	B	0.821	228	100

639	Humerus diaphysis	C	0.810	248	104
639	Humerus epiphysis distal	A	0.804	215	97.5
639	Humerus epiphysis distal	B	0.818	219	94.5
639	Humerus epiphysis distal	C	0.865	213	96.7
639	Humerus epiphysis proximal	A	0.691	192	87.2
639	Humerus epiphysis proximal	B	0.696	193	86.4
639	Humerus epiphysis proximal	C	0.709	215	98.0
639	Tibiotarsus diaphysis	A	0.817	236	104
639	Tibiotarsus diaphysis	B	0.891	234	104
639	Tibiotarsus diaphysis	C	0.871	231	103
639	Tibiotarsus epiphysis distal	A	0.446	94.5	61.2
639	Tibiotarsus epiphysis distal	B	0.421	118	60.0
639	Tibiotarsus epiphysis distal	C	0.583	160	78.9
639	Tibiotarsus epiphysis proximal	A	0.434	142	74.3
639	Tibiotarsus epiphysis proximal	B	0.522	159	73.2
639	Tibiotarsus epiphysis proximal	C	0.414	132	80.4
664	Femur diaphysis	A	23.8	220	101
664	Femur diaphysis	B	22.7	226	101
664	Femur diaphysis	C	22.8	228	103
664	Femur epiphysis distal	A	58.3	178	80.7
664	Femur epiphysis distal	B	92.6	172	77.7
664	Femur epiphysis distal	C	34.5	197	90.8
664	Femur epiphysis proximal	A	39.1	196	89.9
664	Femur epiphysis proximal	B	39.8	195	88.7
664	Femur epiphysis proximal	C	25.1	199	89.8
664	Humerus diaphysis	A	17.8	236	105
664	Humerus diaphysis	B	17.1	231	104
664	Humerus diaphysis	C	16.4	226	101
664	Humerus epiphysis distal	A	23.6	189	85.4
664	Humerus epiphysis distal	B	25.7	185	84.0
664	Humerus epiphysis distal	C	25.7	207	93.1
664	Humerus epiphysis proximal	A	38.5	208	94.8
664	Humerus epiphysis proximal	B	39.2	219	100
664	Humerus epiphysis proximal	C	36.6	223	101
664	Tibiotarsus diaphysis	A	22.9	216	98.0

664	Tibiotarsus diaphysis	B	24.3	239	105
664	Tibiotarsus diaphysis	C	23.3	218	98.5
664	Tibiotarsus epiphysis distal	A	51.2	143	64.0
664	Tibiotarsus epiphysis distal	B	40.4	163	71.9
664	Tibiotarsus epiphysis distal	C	46.7	133	59.4
664	Tibiotarsus epiphysis proximal	A	64.1	175	82.5
664	Tibiotarsus epiphysis proximal	B	71.0	138	63.3
664	Tibiotarsus epiphysis proximal	C	74.1	180	83.9
668	Femur epiphysis distal	A	3.63	337	160
668	Femur epiphysis distal	B	3.09	370	175
668	Femur epiphysis distal	C	8.77	346	163
668	Tibiotarsus epiphysis proximal	A	8.83	202	98.1
668	Tibiotarsus epiphysis proximal	B	12.3	173	83.1
668	Tibiotarsus epiphysis proximal	C	6.12	164	79.4
668	Tibiotarsus diaphysis	A	1.16	250	116
668	Tibiotarsus diaphysis	B	1.13	267	124
668	Tibiotarsus diaphysis	C	0.922	242	111
699	Femur epiphysis distal	A	0.215	191	88.6
699	Femur epiphysis distal	B	0.228	182	83.2
699	Femur epiphysis distal	C	0.232	184	86.2
699	Tibiotarsus epiphysis proximal	A	0.208	150	71.2
699	Tibiotarsus epiphysis proximal	B	0.207	145	68.9
699	Tibiotarsus epiphysis proximal	C	0.149	121	56.3
699	Tibiotarsus diaphysis	A	0.221	233	108
699	Tibiotarsus diaphysis	B	0.209	218	102
699	Tibiotarsus diaphysis	C	0.217	226	106

Table S.2 – Summary of data used in Chapter 3. Monitoring variables and variables derived from blood monitoring data.

Studbook #	Pb Exposure / CoD category	Lifetime integral (µg/dL*year)[i]	Lifetime peak (µg/dL)	18 month integral (µg/dL*year)[ii]	18 month peak (µg/dL)	Free fly days	Age at death (years)	Liver Pb conc. (µg/g)	Feed on proof[iii]
32	NE	NA	NA	NA	NA	NA	31.95	NA	NA
63	Other	39.5	3.00	4.12	3.00	623	23.4	< 1	0.167
102	UKN	85.8	68.0	39.3	68.0	2025	6.50	NA	0.201
112	Pb	472	190	61.3	190	5125	17.0	20.0	0.200
125	Other	604	150	54.7	116	5366	19.1	NA	0.149
192	Pb	442	110	16.1	39.0	5182	17.3	NA	0.162
195	UKN	NA	NA	NA	NA	3263	11.6	NA	0
238	Pb	98.5	80	17.3	80.0	1227	7.12	< 1	0.179
242	Pb	863	610	41.5	165	3634	14.3	83.3	0.156
245	Pb	177	523	143	523	635	6.31	2.00	0.191
246	Pb	NA	NA	NA	NA	3687	10.9	NA	0
265	UKN	NA	NA	NA	NA	2357	13.4	NA	0.025
272	Pb	NA	NA	NA	NA	3804	13.8	NA	0
286	Pb	197	180	142	180	1729	6.99	< 1	0.145
299	UKN	NA	NA	NA	NA	3452	10.7	< 1	0
301	Other	37.6	67.0	33.4	67	878	4.07	< 1	0.106
306	Pb	276	164	7.89	12.0	2809	10.1	52.5	0.184
307	Other	146	130	95.6	130	812	4.03	< 1	0.293
312	Pb	268	250	14.5	14.0	2730	9.92	68.8	0.171
318	Pb	512	760	123	760	2702	9.48	4.60	0.109
345	Pb	220	64.0	31.0	45.1	2568	9.04	42.4	0.184
356	UKN	4.79	3.00	3.79	3.00	472	2.55	NA	0.064
408	UKN	81.8	48.0	16.7	23.0	1111	4.69	NA	0.061

411	UKN	556	200	129	100	1945	8.62	NA	0.219
412	Other	167	220	29.0	65.8	1602	5.74	< 1	0.064
445	NE	NA	NA	NA	NA	NA	2.89	NA	NA
458	Pb	12.7	17.0	10.8	17.0	527	2.81	12.0	0.099
478	Pb	77.9	57.0	24.3	49.3	862	4.00	18.0	0.110
499	UKN	33.4	38.0	30.3	38.0	933	2.64	< 1	0.124
502	NE	NA	NA	NA	NA	NA	2.83	NA	NA
511	Other	NA	NA	NA	NA	NA	1.62	NA	0.174
512	Other	11.1	7.90	7.45	7.90	557	3.74	< 1	0.129
536	UKN	41.2	28.0	29.1	28.0	910	4.28	NA	0.086
553	UKN	NA	NA	NA	NA	NA	2.69	NA	0
598	UKN	42.4	53.0	40.6	53.0	605	1.67	< 1	0.005
615	Other	91.2	69.0	62.6	69.0	130	14.6	NA	0.062
630	Other	26.5	25.0	24.0	25.0	814	2.31	< 1	0.014
639	NE	NA	NA	NA	NA	NA	2.55	< 1	NA
664	Pb	64.8	39.0	45.6	39.0	450	2.65	29.4	0.024
668	UKN	NA	NA	NA	NA	NA	1.86	NA	NA
699	NE	NA	NA	NA	NA	NA	0.67	NA	NA

[i] Area under the graph generated by ploting blood Pb concentration over time, during the lifetime of the condor.

[ii] Area under the graph generated by ploting blood Pb concentration over time, during the 18 months prior to the condor's death.

[iii] Proportion of days free flying observed feeding on proffered carcasses.

Appendix B: Complete data set for Chapter 4

Bellow are the condor cases analysed for chapter 4, but that did not fit the inclusion criteria. Condors 445 and 502 lived in captivity for their whole life and were likely not exposed to elevated amounts of Pb (Table 4.3). For condors 112, 286, and 458, the liver $^{207}Pb/^{206}Pb$ ratio was not measurably different than any of the bone $^{207}Pb/^{206}Pb$ ratios, and condors 245 and 345 had two exposure events during the timeframe of the feather growth (Table 4.3). Therefore, condors 112, 245, 286, 345, 445, 458, and 502 did not fit the criteria to estimate to measure Pb uptake rates.

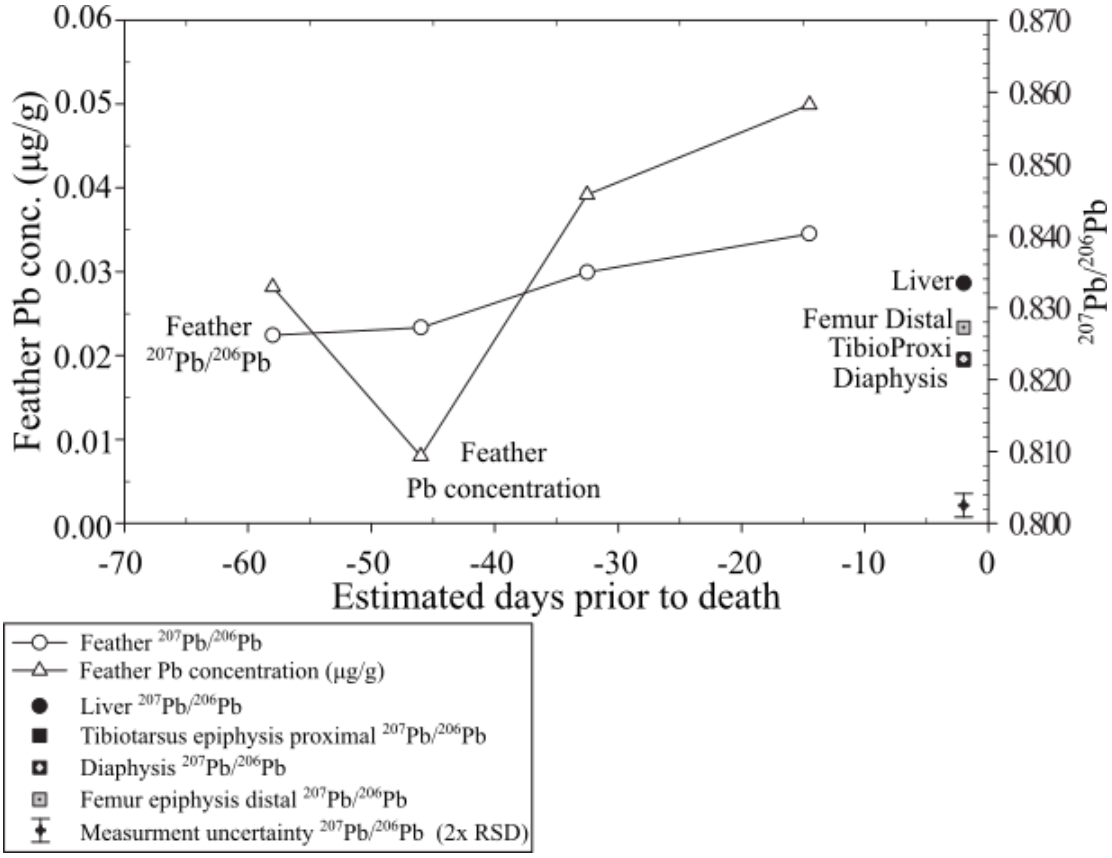

Figure S.1 – Segments from condor 445 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. $^{207}Pb/^{206}Pb$ ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

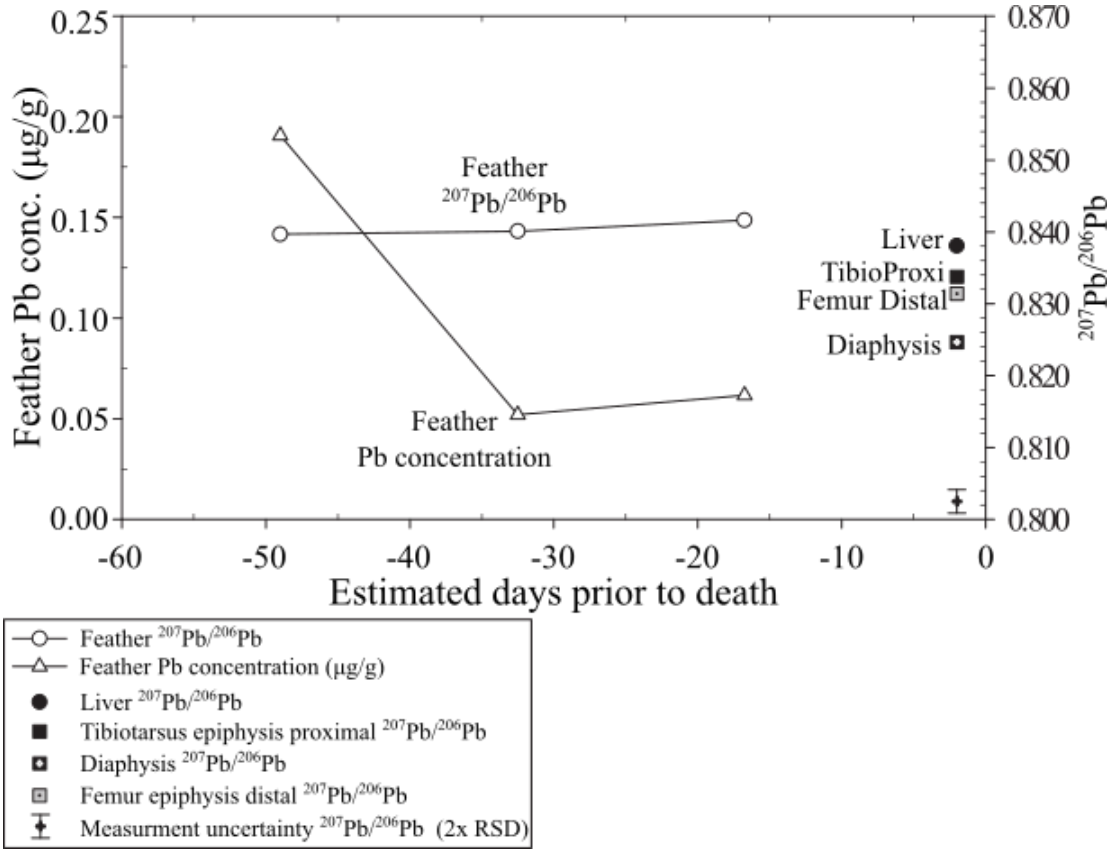

Figure S.2 – Segments from condor 502 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. $^{207}Pb/^{206}Pb$ ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

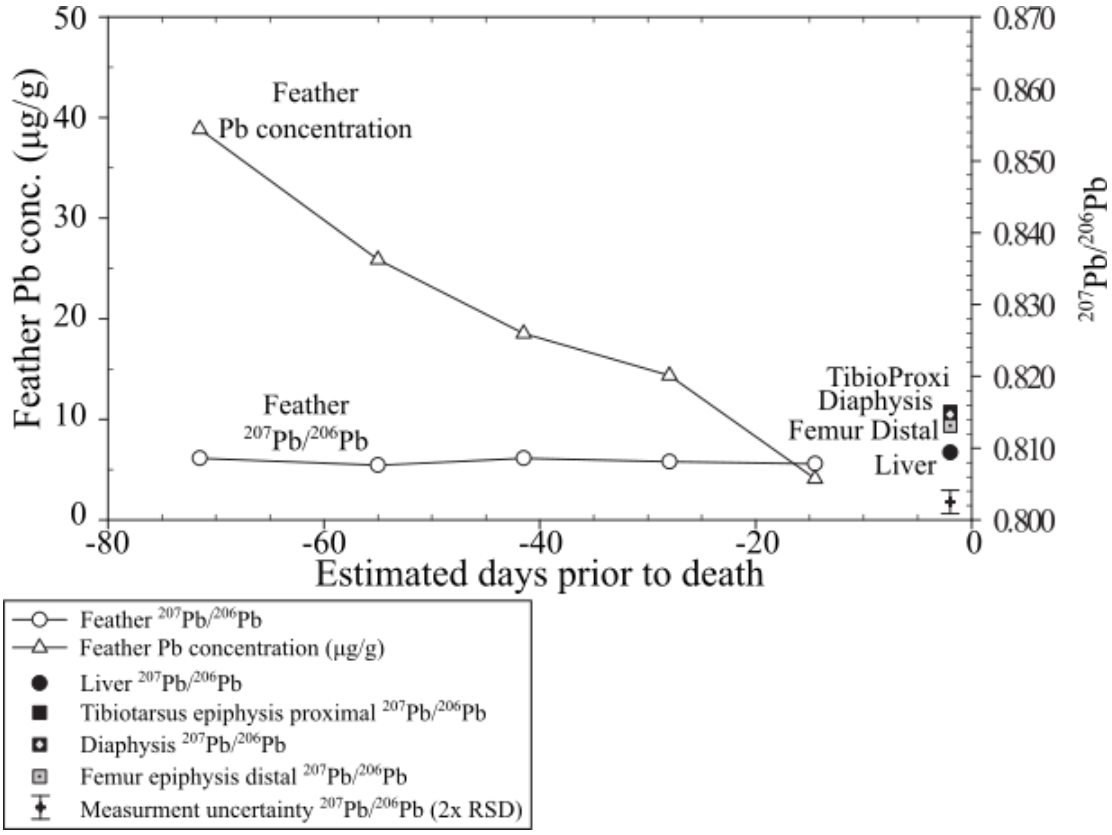

Figure S.3 – Segments from condor 112 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. $^{207}Pb/^{206}Pb$ ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

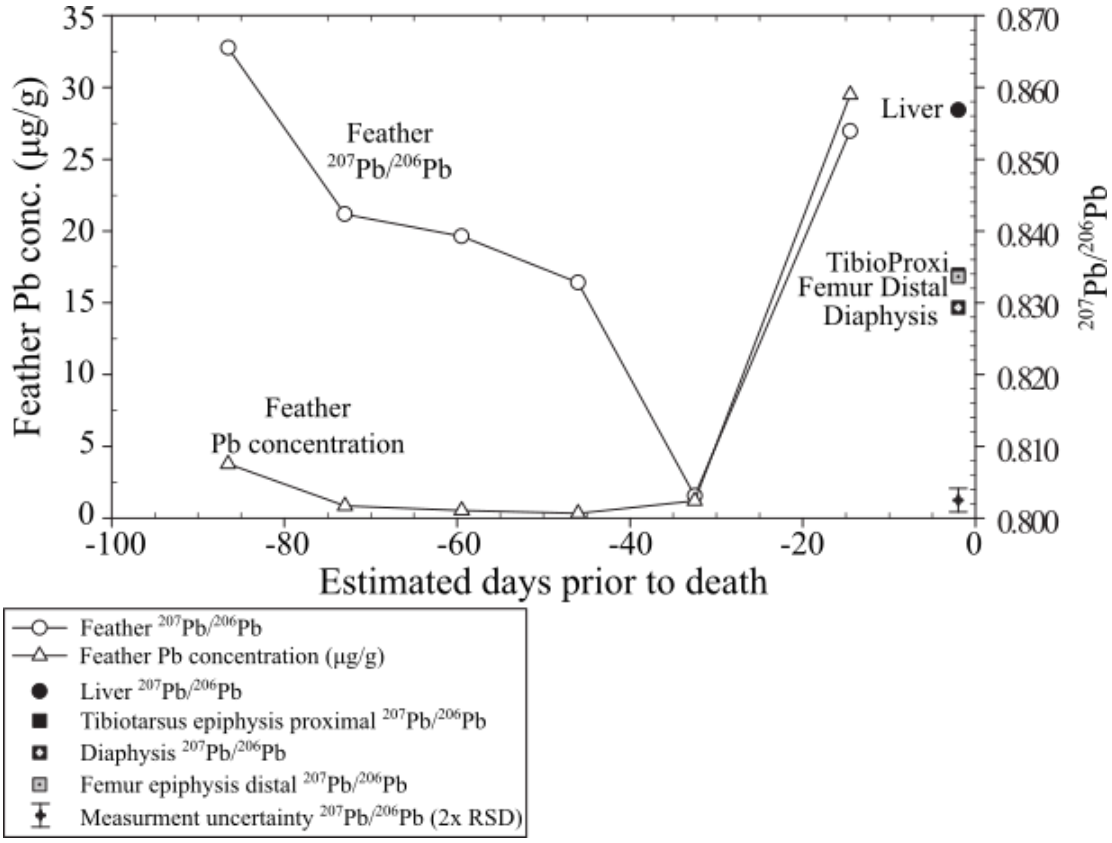

Figure S.4 – Segments from condor 245 growing feather measured for Pb concentration and $^{207}Pb/^{206}Pb$ ratio. The lines represent feather Pb concentration (triangles) and $^{207}Pb/^{206}Pb$ ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. $^{207}Pb/^{206}Pb$ ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

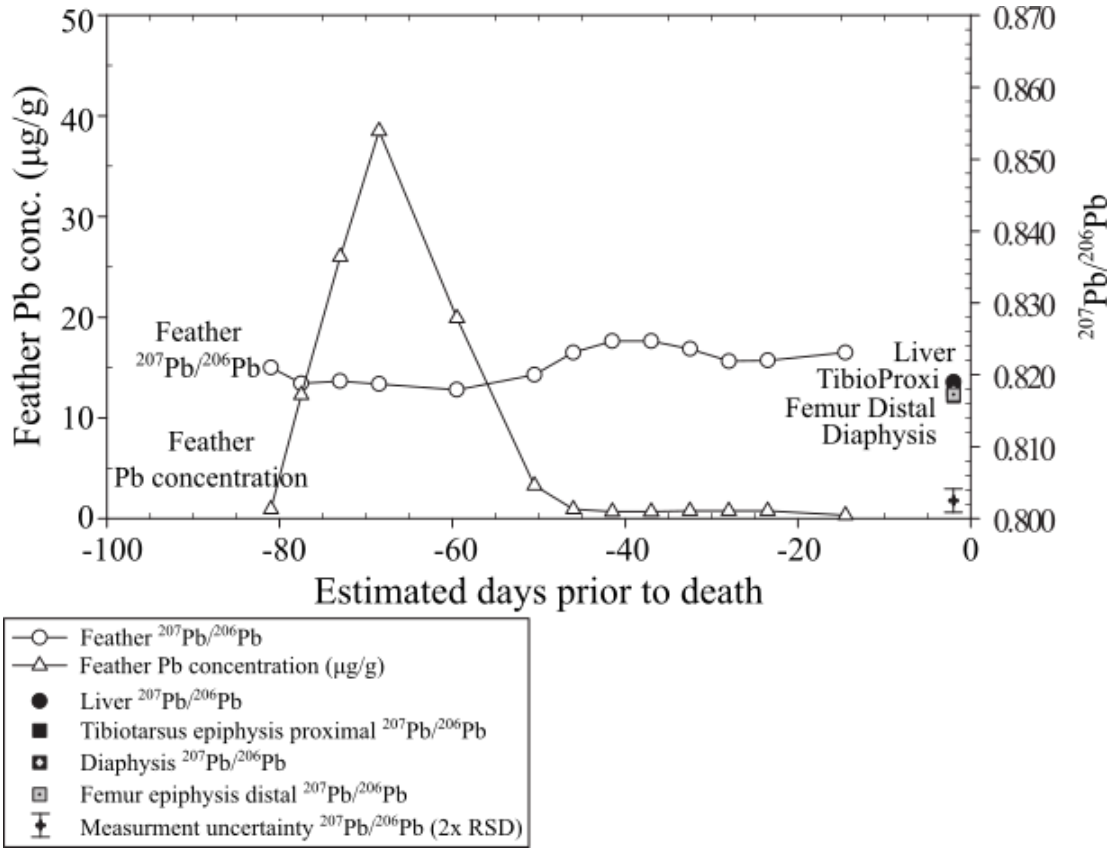

Figure S.5 – Segments from condor 286 growing feather measured for Pb concentration and ^{207}Pb/^{206}Pb ratio. The lines represent feather Pb concentration (triangles) and ^{207}Pb/^{206}Pb ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. ^{207}Pb/^{206}Pb ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

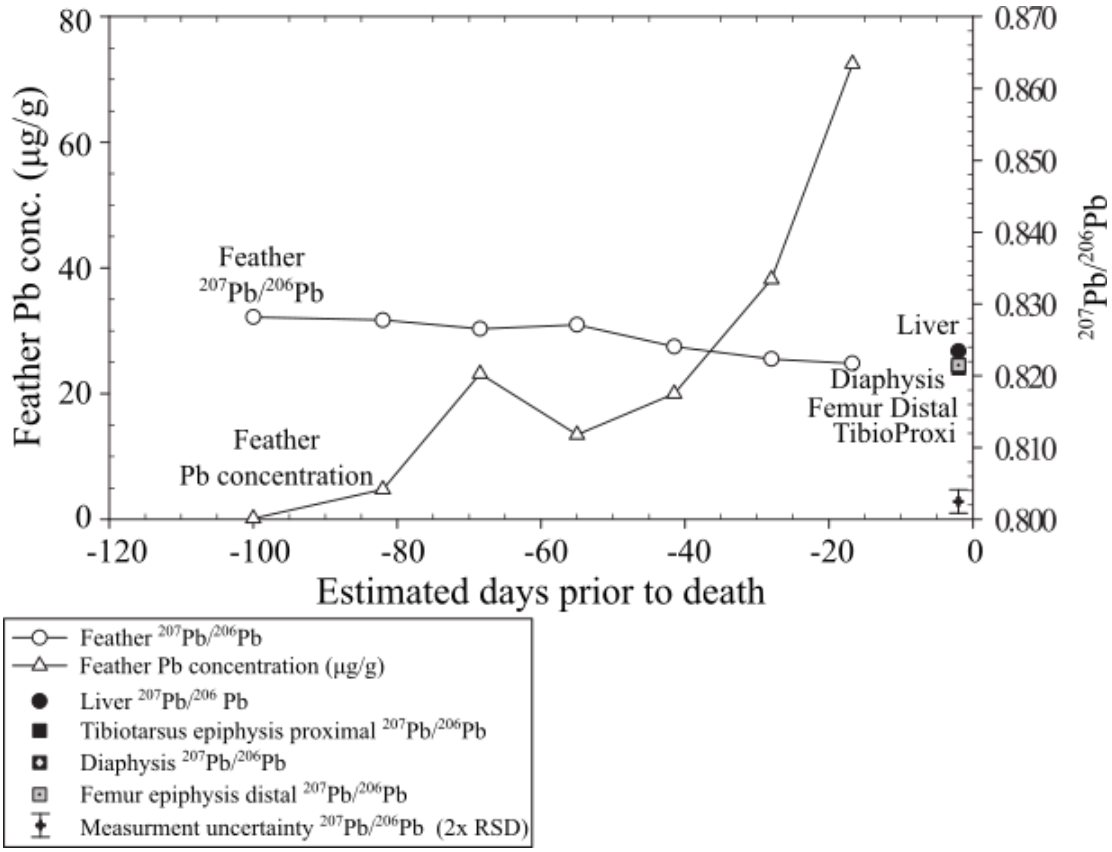

Figure S.6 – Segments from condor 345 growing feather measured for Pb concentration and ^{207}Pb/^{206}Pb ratio. The lines represent feather Pb concentration (triangles) and ^{207}Pb/^{206}Pb ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. ^{207}Pb/^{206}Pb ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

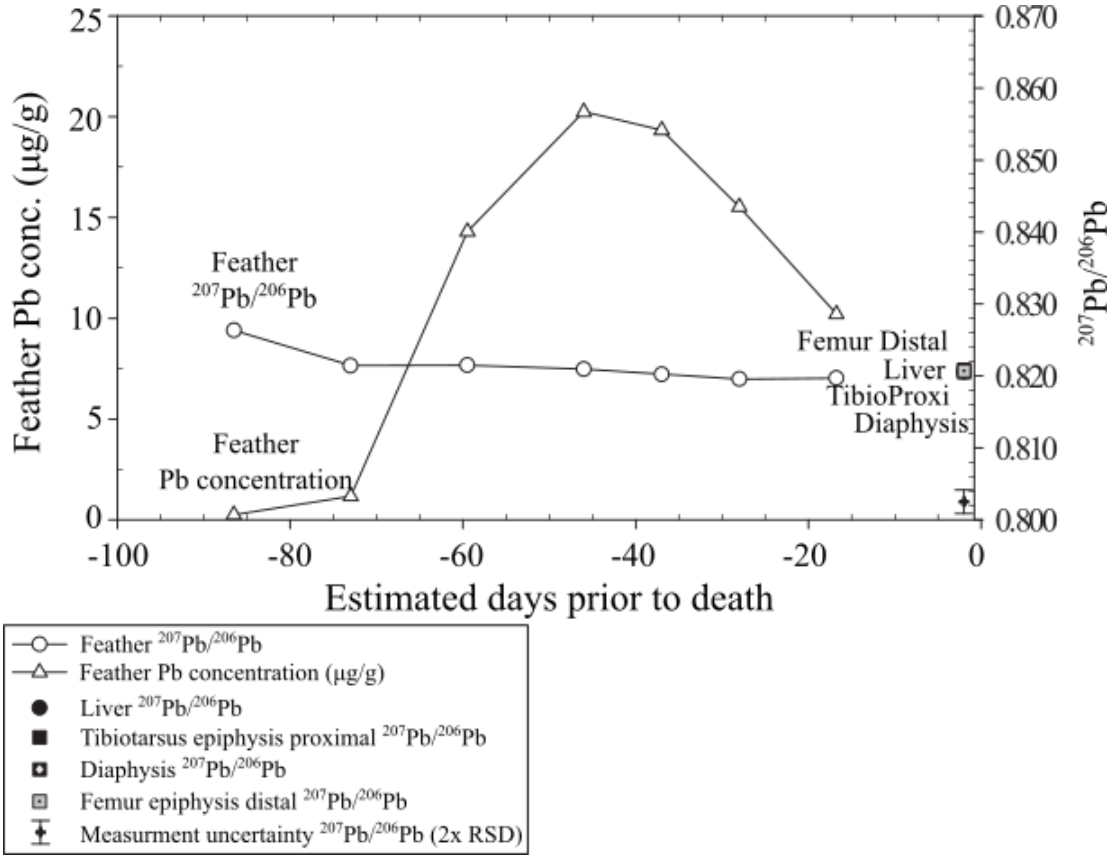

Figure S.7 – Segments from condor 458 growing feather measured for Pb concentration and ^{207}Pb/^{206}Pb ratio. The lines represent feather Pb concentration (triangles) and ^{207}Pb/^{206}Pb ratio (circles) for each feather segment. Each segment is ~2 cm and the feather was collected post-mortem. ^{207}Pb/^{206}Pb ratio isotopic composition for liver (filled circle), tibiotarsus epiphysis proximal (filled square), tibiotarsus diaphysis (dotted square) and femur epiphysis distal (grey square) are also shown. The measurement error (±2x RSD) is represented by the error bar in the bottom left corner of the figure.

Table S.3 – Sample collection information, Pb concentrations, $^{207}Pb/^{206}Pb$ ratios of tissues collected. Also shown are the Pb concentrations and $^{207}Pb/^{206}Pb$ ratios of bone and liver samples, and of the recovered fragment at time of death. Feather and liver were analyzed as single samples and bones were analyzed in duplicate. Bone Pb concentration in Appendix A Table S.1.

Studbook #	**Sample**	**Feather segment # (segment length - cm)** [i]	**Pb concentration (µg/g)**	**$^{207}Pb/^{206}Pb$**	**Estimated blood Pb concentration (µg/dL)**[ii]	**Estimated days prior to death**[i]
445	Feather	P1+P2 (base) (4.0)	0.050	0.8403	0.95	-14.5
445	Feather	P5 (2.0)	0.039	0.8349	0.74	-32.5
445	Feather	P8 (2.0)	0.008	0.8272	0.15	-46
445	Feather	P11+P10 (tip) (3.0)	0.028	0.8262	0.54	-58
445	Liver	-	0.008	0.8334	-	-
445	Tibiotarsus epiphysis proximal A	-	-	0.8234	-	-
445	Tibiotarsus epiphysis proximal B	-	-	0.8220	-	-
445	Tibiotarsus diaphysis A	-	-	0.8223	-	-
445	Tibiotarsus diaphysis B	-	-	0.8235	-	-
445	Femur epiphysis distal A	-	-	0.8278	-	-
445	Femur epiphysis distal B	-	-	0.8267	-	-
502	Feather	P1+P2 (base) (4.0)	0.062	0.8416	1.17	-16.75
502	Feather	P5 (2.0)	0.052	0.8401	0.986	-32.5
502	Feather	P9+P8 (tip) (3.0)	0.19	0.8397	3.63	-49
502	Liver	-	0.16	0.8381	-	-
502	Tibiotarsus epiphysis proximal A	-	-	0.8337	-	-
502	Tibiotarsus epiphysis proximal B	-	-	0.8338	-	-
502	Tibiotarsus diaphysis A	-	-	0.8245	-	-
502	Tibiotarsus diaphysis B	-	-	0.8249	-	-

502	Femur epiphysis distal A	-	-	0.8318	-	-
502	Femur epiphysis distal B	-	-	0.8310	-	-
112	Feather	P1 (base) (2.0)	4.12	0.8078	78.3	-14.5
112	Feather	P4 (2.0)	14.4	0.8081	273	-28
112	Feather	P7 (2.0)	18.6	0.8086	353	-41.5
112	Feather	P10 (2.0)	25.9	0.8076	492	-55
112	Feather	P14+P13 (tip) (3.0)	38.9	0.8086	739	-71.5
112	Liver	-	88.4	0.8094	-	-
112	Tibiotarsus epiphysis proximal A	-	-	0.8156	-	-
112	Tibiotarsus epiphysis proximal B	-	-	0.8144	-	-
112	Tibiotarsus diaphysis A	-	-	0.8146	-	-
112	Tibiotarsus diaphysis B	-	-	0.8147	-	-
112	Femur epiphysis distal A	-	-	0.8134	-	-
112	Femur epiphysis distal B	-	-	0.8128	-	-
238	Feather	P1 (base) (2.0)	6.58	0.8028	125	-14.54
238	Feather	P2 (3.0)	6.76	0.8013	128	-21.34
238	Feather	P3 (2.3)	5.59	0.8044	106	-26.44
238	Feather	P4 (2.7)	2.93	0.8022	55.7	-32.45
238	Feather	P5 (2.8)	1.72	0.8044	32.7	-38.68
238	Feather	P6 (2.6)	0.974	0.8140	18.5	-44.58
238	Feather	P7 (2.8)	0.418	0.8330	7.94	-50.82
238	Feather	P8 (3.3)	0.318	0.8402	6.05	-58.19
238	Feather	P9 (2.9)	0.203	0.8450	3.86	-64.76
238	Feather	P10 (2.5)	0.145	0.8438	2.76	-70.32
238	Feather	P11 (2.8)	0.127	0.8386	2.41	-76.67
238	Feather	P12 (4.2)	0.129	0.8419	2.46	-86.08

238	Liver	-	1.89	0.8053	-	-
238	Tibiotarsus epiphysis proximal A	-	-	0.8385	-	-
238	Tibiotarsus epiphysis proximal B	-	-	0.8371	-	-
238	Tibiotarsus diaphysis A	-	-	0.8448	-	-
238	Tibiotarsus diaphysis B	-	-	0.8434	-	-
238	Femur epiphysis distal A	-	-	0.8463	-	-
238	Femur epiphysis distal B	-	-	0.8462	-	-
245	Feather	P1 (base) (2.0)	29.5	0.8539	560	-14.5
245	Feather	P5 (2.0)	1.20	0.8031	22.9	-32.5
245	Feather	P8 (2.0)	0.336	0.8328	6.39	-46
245	Feather	P11 (2.0)	0.535	0.8393	10.2	-59.5
245	Feather	P14 (2.0)	0.861	0.8424	16.4	-73
245	Feather	P17 (tip) (2.0)	3.77	0.8655	71.7	-86.5
245	Liver	-	5.33	0.8568	-	-
245	Tibiotarsus epiphysis proximal A	-	-	0.8346	-	-
245	Tibiotarsus epiphysis proximal B	-	-	0.8332	-	-
245	Tibiotarsus diaphysis A	-	-	0.8301	-	-
245	Tibiotarsus diaphysis B	-	-	0.8288	-	-
245	Femur epiphysis distal A	-	-	0.8343	-	-
245	Femur epiphysis distal B	-	-	0.8330	-	-
286	Feather	P1 (base) (2.0)	0.330	0.8231	6.27	-14.5
286	Feather	P3 (2.0)	0.740	0.8220	14.1	-23.5
286	Feather	P4 (2.0)	0.760	0.8219	14.4	-28
286	Feather	P5 (2.0)	0.740	0.8236	14.1	-32.5
286	Feather	P6 (2.0)	0.700	0.8247	13.3	-37
286	Feather	P7 (2.0)	0.690	0.8247	13.1	-41.5

286	Feather	P8 (2.0)	0.920	0.8231	17.5	-46
286	Feather	P9 (2.0)	3.30	0.8200	62.7	-50.5
286	Feather	P11 (2.0)	19.9	0.8179	378	-59.5
286	Feather	P13 (2.0)	38.5	0.8187	732	-68.5
286	Feather	P14 (2.0)	26.0	0.8191	494	-73
286	Feather	P15 (2.0)	12.3	0.8188	234	-77.5
286	Feather	P16 (tip) (2.5)	0.930	0.8210	17.7	-81
286	Liver	-	4.07	0.8190	-	-
286	Tibiotarsus epiphysis proximal A	-	-	0.8173	-	-
286	Tibiotarsus epiphysis proximal B	-	-	0.8168	-	-
286	Tibiotarsus diaphysis A	-	-	0.8180	-	-
286	Tibiotarsus diaphysis B	-	-	0.8179	-	-
286	Femur epiphysis distal A	-	-	0.8176	-	-
286	Femur epiphysis distal B	-	-	0.8170	-	-
306	Feather	P1(base) (2.0)	64.9	0.8154	1230	-14.5
306	Feather	P4 (2.0)	42.9	0.8196	816	-28
306	Feather	P7 (2.0)	28.4	0.8235	539	-41.5
306	Feather	P10 (2.0)	5.61	0.8234	107	-55
306	Feather	P13 (2.0)	1.83	0.8231	34.8	-68.5
306	Feather	P17+P16 (tip) (3.0)	2.55	0.8242	48.4	-85
306	Liver	-	207	0.8156	-	-
306	Tibiotarsus epiphysis proximal A	-	-	0.8177	-	-
306	Tibiotarsus epiphysis proximal B	-	-	0.8175	-	-
306	Tibiotarsus diaphysis A	-	-	0.8232	-	-
306	Tibiotarsus diaphysis B	-	-	0.8238	-	-
306	Femur epiphysis distal A	-	-	0.8197	-	-

306	Femur epiphysis distal B	-	-	0.8197	-	-
318	Feather	P1 (base) (2.0)	32.0	0.8271	608	-14.5
318	Feather	P3 (2.0)	57.8	0.8287	1090	-23.5
318	Feather	P5 (2.0)	53.9	0.8281	1020	-32.5
318	Feather	P7 (2.0)	40.9	0.8267	778	-41.5
318	Feather	P9 (tip) (2.0)	21.0	0.8254	400	-50.5
318	Liver	-	15.8	0.8260	-	-
318	Tibiotarsus epiphysis proximal A	-	-	0.8295	-	-
318	Tibiotarsus epiphysis proximal B	-	-	0.8309	-	-
318	Tibiotarsus diaphysis A	-	-	0.8307	-	-
318	Tibiotarsus diaphysis B	-	-	0.8299	-	-
318	Femur epiphysis distal A	-	-	0.8342	-	-
318	Femur epiphysis distal B	-	-	0.8361	-	-
318	Lead fragment	-	-	0.8287	-	-
345	Feather	P1+P2 (base) (4.0)	72.5	0.8217	1380	-16.75
345	Feather	P4 (2.0)	38.2	0.8223	726	-28
345	Feather	P7 (2.0)	20.0	0.8240	381	-41.5
345	Feather	P10 (2.0)	13.5	0.8271	256	-55
345	Feather	P13 (2.0)	23.2	0.8265	440	-68.5
345	Feather	P16 (2.0)	4.80	0.8278	91.2	-82
345	Feather	P20 (tip) (2.0)	0.165	0.8282	3.13	-100
345	Liver	-	147	0.8234	-	-
345	Tibiotarsus epiphysis proximal A	-	-	0.8212	-	-
345	Tibiotarsus epiphysis proximal B	-	-	0.8209	-	-
345	Tibiotarsus diaphysis A	-	-	0.8220	-	-
345	Tibiotarsus diaphysis B	-	-	0.8210	-	-

345	Femur epiphysis distal A	-	-	0.8211	-	-
345	Femur epiphysis distal B	-	-	0.8219	-	-
458	Feather	P1+P2 (base) (4.0)	10.2	0.8197	194	-16.75
458	Feather	P4 (2.0)	15.5	0.8196	295	-28
458	Feather	P6 (2.0)	19.3	0.8202	368	-37
458	Feather	P8 (2.0)	20.2	0.8209	385	-46
458	Feather	P11 (2.0)	14.3	0.8215	272	-59.5
458	Feather	P14 (2.0)	1.16	0.8214	22.1	-73
458	Feather	P17 (tip) (2.0)	0.253	0.8263	4.80	-86.5
458	Liver	-	35.5	0.8206	-	-
458	Tibiotarsus epiphysis proximal A	-	-	0.8210	-	-
458	Tibiotarsus epiphysis proximal B	-	-	0.8200	-	-
458	Tibiotarsus diaphysis A	-	-	0.8212	-	-
458	Tibiotarsus diaphysis B	-	-	0.8205	-	-
458	Femur epiphysis distal A	-	-	0.8204	-	-
458	Femur epiphysis distal B	-	-	0.8210	-	-

[i] Feather vane segments ordered proximal (newest)-distal (oldest) (segment length in cm), PX indicates the xth segement from the feather. Each segment is ~2cm along the rachis axis. Condor feather grow in a constant rate of about 4.4 ± 0.39 mm/day on average and the most proximal feather vane sample is available for collection reflects a condor's blood ~10-20 days beforehand (Church *et al.*, 2006).

[ii] Blood Pb concentrations in µg/dL, feather and tissue Pb concentrations in µg/g dry weight. The relationship between blood lead (µg/dL) and feather lead (µg/g) concentrations (i.e., blood lead:feather lead ratio) is ~19:1 (Finkelstein et al., 2010).