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## Repeated conservation threats across the Americas: High levels of blood and bone lead in the Andean Condor widen the problem to a continental scale<sup>☆</sup>

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## ABSTRACT

Wildlife lead exposure is an increasing conservation threat that is being widely investigated. However, for some areas of the world (e.g., South America) and certain species, research on this subject is still scarce or only local information is available. We analyzed the extent and intensity of lead exposure for a widely distributed threatened species, the Andean Condor (*Vultur gryphus*). We conducted the study at two different scales: 1) sampling of birds received for rehabilitation or necropsy in Argentina, and 2) bibliographic review and extensive survey considering exposure event for the species' distribution in South America. Wild condors from Argentina ( $n = 76$ ) presented high lead levels consistent with both recent and previous exposure (up to 104  $\mu\text{g}/\text{dL}$  blood level, mean  $15.47 \pm 21.21$   $\mu\text{g}/\text{dL}$  and up to 148.20 ppm bone level, mean  $23.08 \pm 31.39$  ppm). In contrast, captive bred individuals -not exposed to lead contamination- had much lower lead levels (mean blood level  $5.63 \pm 3.08$   $\mu\text{g}/\text{dL}$ , and mean bone level  $2.76 \pm 3.06$  ppm). Condors were exposed to lead throughout their entire range in continental Argentina, which represents almost sixty percent ( $>4000$  km) of their geographical distribution. We also present evidence of lead exposure events in Chile, Ecuador, and Peru. Lead poisoning is a widespread major conservation threat for the Andean Condor, and probably other sympatric carnivores from South America. The high number and wide range of Andean Condors with lead values complement the results for the California Condor and other scavengers in North America suggesting lead poisoning is a continental threat. Urgent actions are needed to reduce this poison in the wild.

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## 1. Introduction

Lead poisoning related to human activities has been recognized as a threat for both wildlife and human health worldwide (Watson

et al., 2009; Lambertucci et al., 2010). It has been extensively documented as a conservation problem by studying selected species (Church et al., 2006; Pain et al., 2005, 2009; Fisher et al., 2006), or potentially contaminated territories (Martínez-López et al., 2004; Gómez-Ramírez et al., 2011; Madry et al., 2015). To estimate the actual impact of lead poisoning as a conservation threat, widely distributed species should be surveyed at different spatial scales. Those species could be useful as indicators of a local or a wide range threat that can affect other sympatric species with similar feeding behavior (Gómez-Ramírez et al., 2014).

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Currently the main source of lead available to animals is spent ammunition from hunting activities. (Hunt et al., 2006; Church et al., 2006; Jenni et al., 2015). Among birds, both waterfowl and scavengers are prone to accidental ingestion of lead due to their feeding habits, and therefore much information has been reported from these groups (Guillemain et al., 2007; Friend et al., 2009). Birds of prey have been used as biomonitors for heavy metal exposure since they are at the end of the food chain, and they have large home ranges where they can be exposed to contamination (Dauwe et al., 2003). Lead poisoning, particularly in relation to scavengers, has been poorly studied in South America although hunting has increased in some countries like Argentina during recent decades (Saggeese et al., 2009; Lambertucci et al., 2011; Ferreyra et al., 2014). This situation increases the risk of accidental consumption of lead in the form of wounded animals, non-retrieved carcasses or gut piles left in the field (Hunt et al., 2006), creating a dangerous scenario for obligate scavengers.

Many obligate and facultative scavenger populations are experiencing abrupt declines across the globe (Ogada et al., 2012, 2015). Incidental ingestion of Diclofenac ranks high among main causes for vulture decline (Ogada et al., 2012), but lead poisoning events affecting different vulture species have been documented for North America, Europe and Asia (Fisher et al., 2006; Hernández and Margalida, 2009; Kenny et al., 2015; Nam and Lee, 2009; Pain et al., 2009). In fact, incidental ingestion of spent ammunition from hunted animals is considered the primary factor threatening California Condor (*Gymnogyps californianus*) survival and recovery in the wild (Church et al., 2006; Parish et al., 2009; Finkelstein et al., 2012).

In a given individual the effect of lead contamination can be evaluated not only by lethal effects (Pattee et al., 2006), but also by sub-lethal detrimental effects on physiological functions (Gangoso et al., 2009; Espín et al., 2015), immune system (Franson and Pain, 2011) and behavior (Burger and Gochfeld, 2000). Beyond death of individuals, sublethal long-term effects could play an important role in shaping populations of long lived scavenging species feeding in frequent hunting or contaminated areas. The Andean Condor (*Vultur gryphus*) is a good model species to evaluate the impact of lead exposure in South American carnivores because it is a long-lived top scavenger that feeds mainly on medium-to-large herbivores, many species of which are hunted (Lambertucci et al., 2009a, 2011). Moreover, it is widely distributed throughout the South American Andes, has a low reproductive rate, and depends on high adult survival to maintain a stable population (Lambertucci, 2007). Andean Condor poisoning with lead bullets has been reported and studied in captivity (Locke et al., 1969; Pattee et al., 2006). However, there is a lack of information regarding lead poisoning in wild condors. The only report of lead exposure in free ranging individuals comes from Patagonia, Argentina, through feathers collected in three of the thirteen Argentinean provinces that the species inhabits. Lead concentrations in feathers from condors in those areas were generally low but some individuals were clearly exposed to this metal, with an isotopic signature resembling a mixture of two types of ammunition sources used for local hunting (one used for big game and another for hare hunting, Lambertucci et al., 2011).

In this study, we documented lead poisoning events for the Andean Condor populations by integrating two different scales. First, we determined the geographic extent and intensity of lead exposure in Andean Condors in Argentina (over ca. 60% of the area of distribution of condors), analyzing both previous (bone) and recent (blood) exposure events for each location. We then evaluated lead exposure for the species at a global scale by reviewing scientific and technical reports, and collecting unpublished data coming from surveys conducted in different South American

countries within the species' range.

## 2. Materials and methods

### 2.1. Study species

The Andean Condor (*Vultur gryphus*) is the largest new world vulture. Its sexual dimorphism allows sex determination, and age class can be determined according to feather pattern (del Hoyo et al., 1994). This species inhabits a total North-South distance of ca. 7200 km throughout the Andes Mountains (Ramos, 1999), from northern Venezuela and Colombia to southern Argentina and Chile (del Hoyo et al., 1994, Fig. 1a). Its habitat along the Andes between Argentina and Chile covers ca. 4400 km, around 60% of the total North-South length of its geographical distribution. The Andean Condor is classified as Near Threatened worldwide and it is being negatively affected by several human disturbances (Speziale et al., 2008; Lambertucci et al., 2009b, 2011; BirdLife International, 2016). Northern populations are critically endangered, and it is considered Vulnerable in Chile and Argentina (Glade, 1988; López-Lanús et al., 2008). Its low genetic diversity (Hendrickson et al., 2003), retraction in its distribution range and local extinction events for South America could lead to population isolation and fragmentation. Southern populations are probably the largest (Lambertucci, 2010), but they show signs of retractions (Lambertucci, 2007; BirdLife International, 2016).

### 2.2. Sample collection for lead exposure assessment in Argentina

When possible lead concentrations were measured both in blood and bone samples, when possible, using protocols certified by a local ethics committee (CICUAL No. 2013/44, School of Veterinary Medicine, University of Buenos Aires). We surveyed lead in blood to know recent exposures, because lead is cleared relatively rapidly from blood with an estimated elimination half-life of 13 days (Finkelstein et al., 2012). On the contrary, lead stored in bones is considered the best indicator for evaluating lead exposure over the total lifetime (Franson and Pain, 2011).

To analyze background lead concentration for Argentine condors, we sampled 10 captive reared chicks (5 males and 5 females) between 6 and 9 months of age (See Table S1 Supplementary Material). Those condors with no history of previous lead exposure events were housed at Buenos Aires Zoo and fed with a controlled diet (certified bovine meat and lab rodents).

Recent lead exposure was determined through blood samples (from 10 to 20 ml, never exceeding 1% body mass). After bleeding from the metatarsal or cubital vein, 0.5–1 ml aliquots of heparinized blood were refrigerated and sent to the laboratory for lead determination. Bone stored lead was analyzed from live birds through bone biopsies from the proximal tarsus-metatarsus. Bone biopsies were performed with minimally invasive techniques under general anesthesia following surgical aseptic protocols and using an 8G Jamshidi Biopsy Needle. Birds handling and sampling protocols were certified by ethics committee approval (CICUAL No. 2013/44).

We analyzed a total of 76 free ranging individuals from Argentina (42 males including 6 juveniles, 7 subadults, 28 adults and 1 non available data; 34 females including 10 juveniles, 3 subadults and 21 adults) that either arrived at the Buenos Aires Zoo facilities for rehabilitation ( $n = 62$ ) or were submitted for necropsy ( $n = 14$ ). Those individuals came from all 13 continental provinces of Argentina within the species' area of distribution, and were admitted between 2008 and 2014. We recorded lead concentrations for each bird in both blood and bone tissue, linking these results to the bird's trapping site (as a proxy for province of origin).

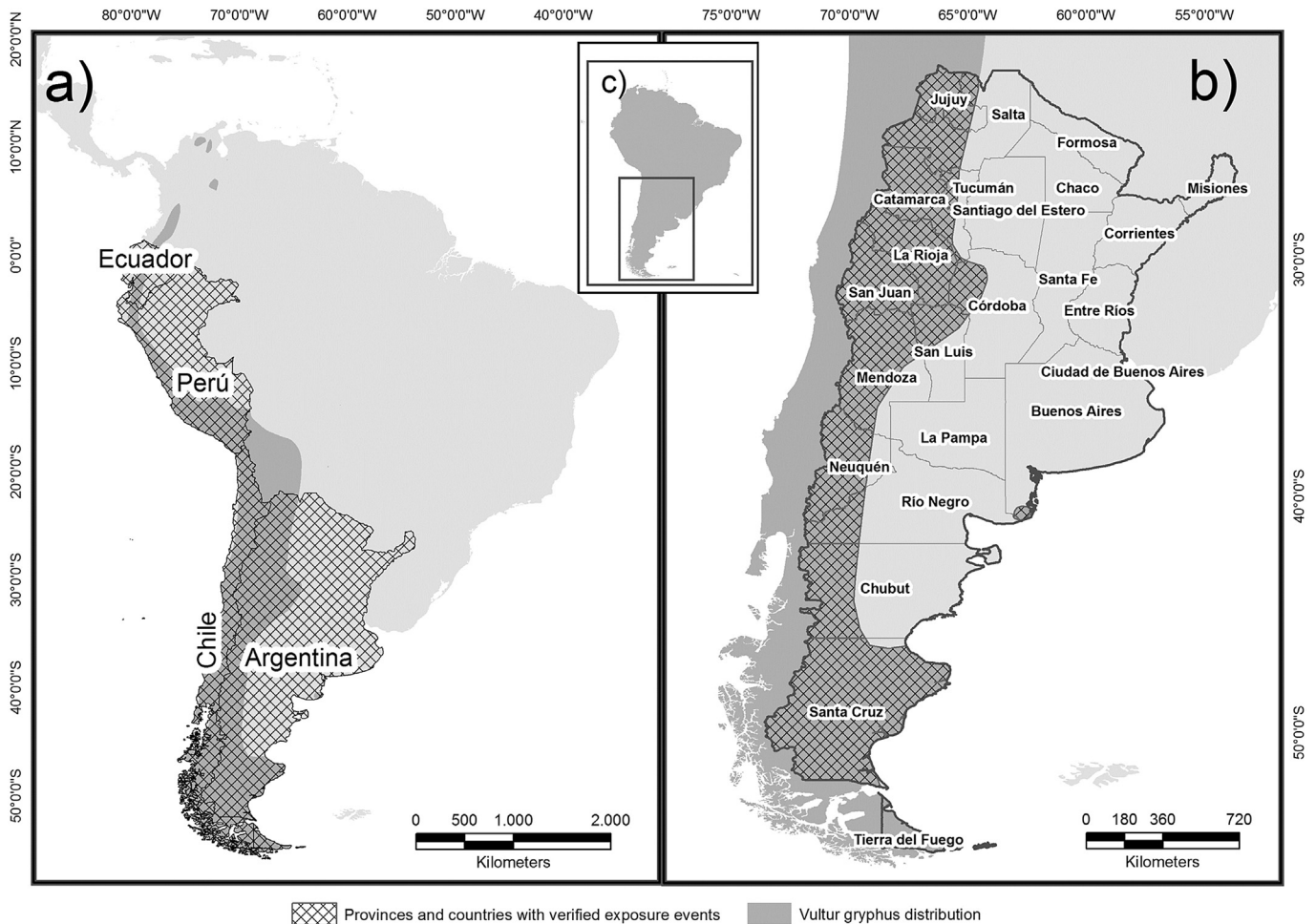


Fig. 1. Andean Condor distribution and events of lead exposure documented for a) South America (countries) and b) Argentina (provinces).

Birds were classified according to sex and age (juveniles, subadults and adults) based on external sexual dimorphism and feather pattern (see Study species). Additional information concerning presence/absence of lead pellets or gunshots in X-ray examination was also recorded when possible (see Table S2, Supplementary information). We discriminated if the pellet was embedded in a tissue (body, legs or wings) or located inside the gastrointestinal tract lumen (GIT).

Recent lead exposure was determined for 62 individuals through blood samples (following same procedure mentioned for captive bred condors) during the first 48 h after admittance. Bone stored lead was analyzed in 47 samples (33 corresponded to bone biopsies from the proximal tarsus-metatarsus taken from alive birds, and 14 corresponded to samples from necropsies). Post mortem bone samples were obtained from dead birds submitted to Buenos Aires Zoo from different locations in Argentina. When the leg bone was not available, large bones from the wing were sampled ( $n = 3$ ). Handling and sampling protocols were performed under the same certified protocols approved by the ethics committee mentioned above.

### 2.3. Lead determination in Argentina

Heparinized blood was digested by mixing a sub-sample of 100  $\mu$ l blood with 900  $\mu$ l 1% TRITON X-100 (w/v). Bone samples were pretreated with acetone, then gently washed with 1% sodium

hydroxide and double-distilled water and dried in oven at 100–105 °C. Bone ashes were treated with 2 ml 60% nitric acid and submitted to a progressive thermal treatment. Once dried, samples were left to cool and suspended in ultrapure distilled water. Lead concentration was determined following methodology of Shah et al. (2010) with a Perkin–Elmer atomic absorption spectrometer model A. Analyst 700 equipped with a flame burner, a graphite furnace HGA-400 pyrocoated graphite tube with integrated platform, an autosampler AS-800 and deuterium lamp as background correction system. A single-element hollow cathode lamp of Pb (Perkin Elmer) was used. Portions of both, standard/sample and modifier were transferred into the auto-sampler cups to inject a 20- $\mu$ l sample (standard or sample volume 10  $\mu$ l + 10  $\mu$ l modifier in each case). Before each analysis round, equipment was calibrated from Pb standard solutions of 1000 mg/L. Detection limit was 0.12  $\mu$ g/dL with a recovery rate of 96.53%. Blood lead concentration was then expressed in  $\mu$ g/dL and bone lead expressed on a dry weight basis (ppm).

### 2.4. Lead exposure in South America

Lead exposure events in other South American countries were documented through an extensive survey of scientific literature, interviews of investigators from every country inside the species' distribution, contacting major zoological institutions, rehabilitation centers, universities, conservation institutions, NGO reports, and



other grey literature. Lead levels from blood, feathers, bone, liver and kidney were considered, as well as X-rays showing lead pellets in condor digestive tract or embedded in body tissues. Biases may exist when comparing lead levels obtained through different techniques, equipment and operators. To ensure reliability and potential comparative results, reports from other countries than Argentina were considered when lead levels were determined by internationally accepted equipment (e.g., fast portable analyzers, usually used by rehabilitation centers), national reference level institutions (certified laboratories or national health institutes) or higher education institutions (teaching hospitals, colleges and universities). Differences in units from different reports were standardized by converting all the data to  $\mu\text{g}/\text{dL}$  or ppm following Franson and Pain (2011).

### 2.5. Lead exposure criteria

Threshold levels at which enzymatic inhibition and other deleterious effects caused by lead are produced, have been reported for a variety of free-living bird species. In a recent study Espín et al. (2015) found more than 15%  $\delta$ -ALAD inhibition (and increased oxidative stress biomarkers) for Griffon Vultures (*Gyps fulvus*) with blood lead above 10  $\mu\text{g}/\text{dL}$ . Finkelstein et al. (2012) reported California Condors with blood lead  $\geq 20$   $\mu\text{g}/\text{dL}$  considered to have significant subclinical health effects (>60% inhibition of the  $\delta$ -ALAD enzyme), and serious clinical effects and need for clinical intervention to avert morbidity and mortality when blood lead levels exceeded 45  $\mu\text{g}/\text{dL}$ . Thus, as we worked with wild condors that were found in the field with some illness, apart from obtaining reference values for captive-reared condors, we also considered categories according to literature information on lead exposure from wild individuals of similar species (Table 1). We note that in this work we did not analyze relationships between clinical signs or subclinical indicators in condors and their lead values, since the information collected does not allow such comparisons.

### 2.6. Data analysis

We used descriptive statistics (mean, median, standard deviation, minimum and maximum) to show values and variability of blood and bone lead levels in relation to sex, age, trapping location (consigned as province) or evidence of lead ammunition inside the body (X-ray examination), and compared them using nonparametric statistics (Kruskal-Wallis test, Conover, 1980). A simple regression was used to evaluate if blood lead concentration was related to bone lead concentration. We then evaluated the potential influences of the variables measured on blood and bone lead levels using generalized linear models (GLM; Normal Distribution; when needed we log-transformed the data to obtain normality) (McCullagh and Nelder, 1989). For this, we built a model including

sex, age, presence of ammunition and origin (consigned as province) as explanatory variables and blood or bone lead levels as the dependent variable.

## 3. Results

### 3.1. Background and reference levels for Argentina

All captive-reared individuals sampled to obtain reference levels presented detectable lead in both blood and bone tissue (Table S1, Supplementary Material). Blood lead concentrations from those individuals were generally low (mean: 5.63  $\mu\text{g}/\text{dL}$ ; SD:  $\pm 3.08$ ) and ranged from 0.02 to 9.20  $\mu\text{g}/\text{dL}$  (i.e., below the 10  $\mu\text{g}/\text{dL}$  threshold considered for background exposure in the literature). Bone values ranged from 0.40 to 8.70 ppm with a mean of 2.76 ppm (SD:  $\pm 3.06$ ), also below the 10 ppm threshold for background exposure described in the literature.

### 3.2. Lead in wild condors from Argentina

All the 76 individuals sampled presented detectable lead levels (Table S2, Supplementary Material). Blood lead ranged from 0.02 to 104  $\mu\text{g}/\text{dL}$ , with a mean of  $15.47 \pm 21.21$   $\mu\text{g}/\text{dL}$  ( $n = 62$ ). Regarding exposed birds 9 of 62 (14.5%) condors presented values between 10 and 20  $\mu\text{g}/\text{dL}$  which has been associated by previous investigations with mild to moderate subclinical effects on health; 8 of 62 (13%) presented values between 20 and 45  $\mu\text{g}/\text{dL}$  suggesting significant subclinical health effects; and 5 of 62 (8%) birds presented values  $\geq 45$   $\mu\text{g}/\text{dL}$  suggesting the need for clinical intervention to avert morbidity and mortality (Table 1). From X-ray examination we found that 15 of 62 (24.2%) condors surveyed presented fragments of lead ammunition inside the body (Table S2, Supplementary Material). Two animals had ammunition in the gastrointestinal tract, and the remaining 13 had ammunition embedded in different body tissues (See Fig. 2).

Bone lead ranged from 1.30 to 148 ppm with a mean of  $23.08 \pm 31.39$  ppm ( $n = 47$ ) (see Fig. 3). These high bone lead values are up to approximately ten times above mean local references from unexposed captive-reared condors. This suggests that condors are victims of heavy lead exposure over a long-term perspective. Forty percent of samples (19 of 47) were below background levels (10 ppm), while 28 of 47 (60%) individuals were exposed according to the aforementioned categories (Table 1): 28% had bone lead values consistent with subclinical to clinical poisoning (between 10 and 20 ppm), and 32% had bone lead values suggesting severe clinical poisoning ( $\geq 20$  ppm). Birds from all the species' range in Argentina presented detectable blood and bone stored lead (Fig. 1b). Mean bone lead levels were above 10 ppm (background levels) for 85% of provinces (11 of 13), confirming long-term exposure with values as high as ten times the background reference (e.g., La Rioja,

**Table 1**  
Categories of lead exposure used to classify the degree of clinical affection and summary of the results (in proportion and percentage) for the Andean condors from Argentina.

Tissue	Value	Interpretation	n (%)
Blood	<10 $\mu\text{g}/\text{dL}$	Background <sup>a</sup>	40/62 (64.5%)
	10–20 $\mu\text{g}/\text{dL}$	Mild to moderate subclinical effects on health <sup>a</sup>	9/62 (14.5%)
	20–45 $\mu\text{g}/\text{dL}$	Significant subclinical health effects <sup>b</sup>	8/62 (13%)
	>45 $\mu\text{g}/\text{dL}$	Need for clinical intervention to avert morbidity and mortality <sup>b</sup>	5/62 (8%)
Bone	<10 ppm	Background <sup>c</sup>	19/47 (40%)
	10–20 ppm	Subclinical to clinical poisoning <sup>c</sup>	13/47 (28%)
	>20 ppm	Severe clinical poisoning <sup>c</sup>	15/47 (32%)

n = number of individuals presenting that had lead concentration in each category from the total.

<sup>a</sup> Sensu Espín et al., 2015.

<sup>b</sup> Sensu Finkelstein et al., 2012.

<sup>c</sup> Sensu Franson and Pain, 2011.

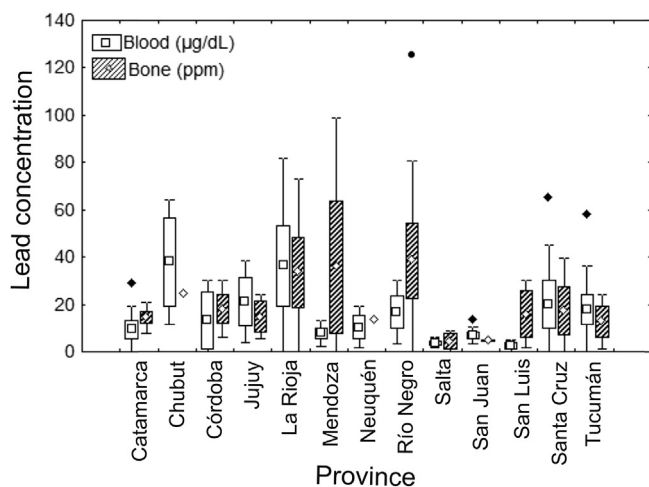


**Fig. 2.** Photo and X-ray of a male condor with lead ammunition in the comb and the face (above). Photo of a condor being X-rayed (below left), and two X-rays of condors with lead bullets in their crop (center) and proventriculus (right). Bullets are highlighted with red circles. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Mendoza and Río Negro, see [Supplementary Material Table S3](#)).

There were no clear differences in blood lead between sexes (Kruskal-Wallis  $H(1/62) = 0.02$ ;  $p = 0.887$ ). Blood lead concentrations differed by age classes, with adults being more exposed than immature birds (juveniles plus subadults) ( $H(1 \text{ of } 62) = 3.34$ ;  $p = 0.048$ ). All birds were recently exposed to lead regardless of province of origin (evidenced by blood levels; [Fig. 1b](#)). Blood lead was higher than  $10 \mu\text{g/dL}$  in 62% (8 of 13) of analyzed territories ([Table S3](#)), but lead concentrations did not differ significantly between provinces ( $H(12 \text{ of } 62) = 18.44$ ;  $p = 0.103$ ).

We found a low but significantly positive relationship between



**Fig. 3.** Blood and bone level concentrations for Andean Condors received for rehabilitation from different provinces in Argentina (see [Fig. 1](#) for the location of each province). We present the Mean  $\pm$  SE (box), Standard Deviations (Whisker) and extreme values (black diamond for blood, and circle for bone).

bone lead levels and blood lead levels ([Fig. 4](#),  $R^2 = 0.21$ ,  $p = 0.008$ ). Birds with lead ammunition in their bodies based on X-ray examination ( $n = 15$ ) showed much higher mean lead concentrations (mean for bone 30.89 vs 16.17 ppm; mean for blood 24.04 vs. 12.81  $\mu\text{g/dL}$ ), but there were no statistical differences between these two groups bone lead levels  $H(1/34) = 0.2057$ ;  $p = 0.650$ ; blood ( $H(1/60) = 1.60$ ;  $p = 0.205$ ).

Nevertheless, GLM models showed that blood lead levels were significantly influenced by age ( $p = 0.012$ ), the presence of ammunition ( $p = 0.018$ ) and geographical location ( $p = 0.006$ ) when all factors were considered together ([Table 2](#)). Among specific locations, La Rioja ( $p < 0.001$ ) and Jujuy ( $p = 0.05$ ) significantly influenced blood lead levels ([Table S4](#), [Supplementary Material](#)).

### 3.3. Lead exposure in South America

There is no published information in scientific journals regarding lead levels in the Andean condor across South America. Existing references come from technical reports or personal communications. In Ecuador, [Ortega et al. \(2010\)](#) documented blood lead values for a group of 19 captive condors, most of them originally admitted for rehabilitation or confiscated. Values ranged from 2 to 45  $\mu\text{g/dL}$  with a mean value of 20.79  $\mu\text{g/dL}$  ( $\pm$ SD 11.89  $\mu\text{g/dL}$ ). Seven of 19 individuals had gunshot ammunition (between 1 and 59 gunshot pellets) embedded in their body based on X-ray examination ([Ortega et al., 2010](#)). In Peru, a health survey including nine condors admitted for rehabilitation near the city of Lima indicated detectable levels in 6 of 9 birds, with a mean of 9.95  $\mu\text{g/dL}$  and values ranging from 3.7  $\mu\text{g/dL}$  to 17.4  $\mu\text{g/dL}$ . The highest value corresponds to a condor admitted due to a gunshot wound, presenting 45 lead pellets embedded in body tissues as confirmed by X-ray examination (L. Schaefer pers. com.). In Chile, lead poisoning events after ammunition ingestion were documented in the southern region of Los Lagos during 2012. Two wild condors were

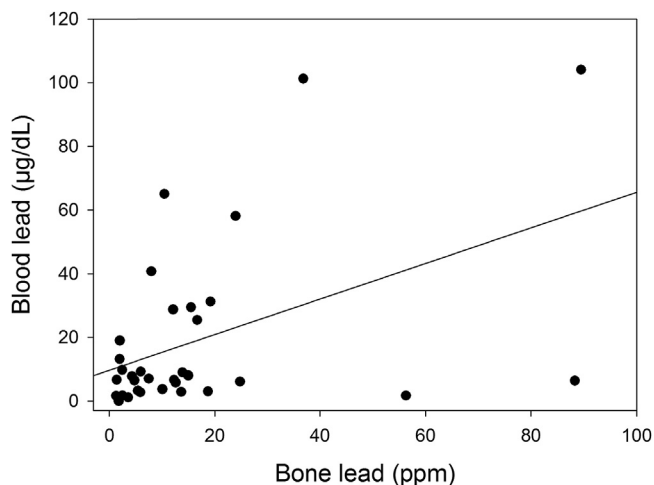


Fig. 4. Relationship between blood and bone lead levels for Andean Condors in Argentina.

admitted at a University Teaching Hospital located in Puerto Montt, after ingesting lead bullets with high levels in blood (89 µg/dL) for a young male, and high levels in liver and kidney (136 and 247 ppm respectively) for an adult female (M. Cabrera et al., unpublished data).

#### 4. Discussion

We documented recent and previous Andean Condor lead exposure for every Argentine continental province covering more than 4000 km South-North, almost 60% of the species distribution. Considering that condors frequently cross political and geographical borders between Argentina and Chile (Lambertucci et al., 2014), but also between Argentina and Bolivia (De Martino, 2009), lead exposure registered in Argentina might be similar in neighboring countries. Worryingly, even though scientific reports for other countries in Latin America are scarce, our additional data on exposure events documented for southern Chile, and results of lead concentration for birds from Peru and Ecuador support this fact. This evidence suggests for the first time that lead poisoning is not a locally restricted problem for some populations of a top scavenger as the Andean Condor, but a widespread conservation threat throughout most of its distribution range in South America (Fig. 1a).

Our samples come from individuals submitted for rehabilitation or from dead animals, thus they are a subset of the population that has been suffering some illness and not a random sample of the wild population. However, lead exposure in Andean Condors has been previously evaluated in a random sample of free-ranging individuals through noninvasive samples (feathers) in the north-western of Argentine Patagonia (Lambertucci et al., 2011). That wild population presented low mean lead levels (1.7 ppm), although some individuals were exposed to high levels of lead

Table 2

Relationship (Generalized linear model with normal distribution) between blood lead levels and geographical location (province), bird age and lead ammunition inside body. Significant values are in bold.

Variable	DF	Wald	P
Intercept	1	27.153	<0.001
Province	12	27.681	<b>0.006</b>
Age	1	6.243	<b>0.012</b>
Ammunition in body	1	5.566	<b>0.018</b>
Age*Ammunition in body	1	0.726	0.393

concentrations, several times above the overall mean (up to 21.1 ppm, Lambertucci et al., 2011). Our work support and extends those previous results showing that recent exposure to lead above the background level can be frequently documented in almost their entire distribution in Argentina, with 35.5% of individuals received for rehabilitation exceeding the background. Therefore, we call for regional alliances for the conservation of this species and highlight the need to adopt regional research goals in order to fulfill information gaps on threats as lead poisoning.

For the first time we confidently demonstrated the relevance of ammunition in condors from Argentina, with 25% of sampled individuals presenting lead fragments in X-ray examinations (embedded in different tissues or in gastrointestinal tract). Most of those birds have embedded ammunition, which highlight the problem of condor illegal persecution (Lambertucci, 2007; BirdLife International, 2016; Williams et al., 2011). The evidence for projectiles inside the digestive tract was low, probably due to the fact that such exposure results in acute toxicosis and sudden death in the field, or because lead fragments could be eliminated through pellet regurgitation or feces after contaminating the bird (but see Pattee et al., 2006). Thus, our results support the fact that condors can be contaminated because of direct persecution or incidental ingestion of gunshot from non-retrieved hunting carrion.

We found a positive association between age and blood lead levels that could be related to their movement patterns. Adults and immature condors have different habitat use (De Martino, 2009; Lambertucci et al., 2014; authors unpublished data), thus, they have different probabilities of being exposed to threats. However this result could also be influenced by the high longevity of adults which can be exposed to sublethal lead levels during many years (Gangoso et al., 2009). Anthropogenic disturbances may have a stronger effect on female and juvenile condors because they can be segregated to areas closer to humans increasing the risk of persecution (Donazar et al., 1999; Lambertucci et al., 2012). However, we found no relationship between blood lead levels and the sex of the individuals, being both males and females similarly affected.

The mean blood lead value of surveyed condors was consistent with those suggested to cause subclinical deleterious effects on California Condors health (Finkelstein et al., 2012), and some individuals reached up to 104 µg/dL. The low positive, but significant association of high blood lead levels with high bone lead levels suggests recirculation of lead between bone and blood (Berlin et al., 1995). The high levels of lead in bone (mean 23.08 ppm) suggest severe clinical poisoning sensu Franson and Pain (2011). Values of sublethal lead exposure (recent, previous or chronic) registered for the studied population in Argentina represents a dangerous scenario. The accumulation of toxins at subclinical levels could be the cause of immunosuppression, increasing susceptibility to infectious diseases and oxidative stress biomarkers (Blanco et al., 2004; Daszak et al., 2001; Dobson and Foufopoulos, 2001; Espín et al., 2015; Snoeijs et al., 2004). Lead could also be playing an important role in lowering the reproductive rate because it decreases sperm quality and consequently fertilization success (Benoff et al., 2000; Castellanos et al., 2008). Importantly, lead accumulated in bones of females could be mobilized during eggshell formation (Taylor, 1970) and transmitted to both the eggshell and to the embryo (Edens and Garlich, 1983; Grandjean, 1976; Pattee, 1984).

Sublethal lead concentrations can be fixed in bone tissue, affecting bone mineralization and increasing bone fractures (Gangoso et al., 2009). This stored lead could then also be released to the bloodstream during physiological periods or metabolic conditions with high bone turnover or resorption (Carmouche et al., 2005), as happen during the healing of fractures (Berlin et al., 1995), malnutrition or prolonged fasting (G. Wiemeyer,



unpubl. data). Thus, endogenous lead mobilization from bones to bloodstream could be causing condors poisoning up to many years after original exposure events, highlighting the importance of bone testing not only during necropsy procedures but in live birds. Further investigations are needed to detect lead levels that can be associated with subclinical affections of reproductive performance, physiology, and immune and nervous system.

Lead poisoning is threatening vultures conservation in different continents (e.g., Gangoso et al., 2009; Kenny et al., 2015; Hernández and Margalida, 2009; Nam and Lee, 2009). Our results in accordance with those reported for California condors and other scavengers from North America (Kelly and Johnson, 2011; Johnson et al., 2013; Behmke et al., 2015), suggest lead is threatening scavengers across the Americas. Among New World scavengers, the California Condor is the species most affected by lead toxicosis, and lead is still considered a key factor holding back the recovery of this critically endangered species (Finkelstein et al., 2012). There are several differences between both Andean and California Condors, particularly regarding population size and geographical distribution. Nevertheless, they share a high exposure to lead with similar proportions of birds showing blood values that suggest subclinical health effects (35% for this study, against 30% reported for the California Condor, Finkelstein et al., 2012). Despite the fact that the Andean Condor population is far larger and probably healthier than that of California Condor, similar subclinical exposure degrees for the Andean Condor populations call to urgently consider this threat to avoid a catastrophic collapse such as that suffered by the California Condor.

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### Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.envpol.2016.10.025>.

### References

Behmke, S., Fallon, J., Duerr, A.E., Lehner, A., Buchweitz, J., Katzner, T., 2015. Chronic lead exposure is epidemic in obligate scavenger populations in eastern North America. *Environ. Int.* 79, 51–55.

Benoff, S., Jacob, A., Hurley, I.R., 2000. Male infertility and environmental exposure to lead and cadmium. *Hum. Reprod. Update* 6, 107–121.

Berlin, K., Gerhardsson, L., Börjesson, J., Lindh, E., Lundström, N., Schütz, A., Edling, C., 1995. Lead intoxication caused by skeletal disease. *Scand. J. Work Environ. Health* 21, 296–300.

BirdLife International, 2016. *Vultur gryphus*. The IUCN Red List of Threatened Species. Version 2015.2. Downloaded on 30th February 2016. <http://www.iucnredlist.org>.

Blanco, G., Jimenez, B., Frias, O., Millan, J., Davila, J.A., 2004. Contamination with nonessential metals from a solid-waste incinerator correlates with nutritional and immunological stress in prefledgling black kites (*Milvus migrans*). *Environ. Res.* 94, 94–101.

Burger, J., Gochfeld, M., 2000. Effects of lead on birds (Laridae): a review of laboratory and field studies. *J. Toxicol. Environ. Health Part B* 3, 59–78.

Cabrera, M., Ojeda, J., Barrios, N., Vera, F., Alvarado, M., Quiróz, E. report First Clinical Report of Lead Poisoning Caused by Ingestion of Shots in Wild Andean Condor (*Vultur gryphus*). Unpublished results.

Carmouche, J.J., Puzas, J.E., Zhang, X., Tiyapatanaputi, P., Cory-Slechta, D.A., Gelein, R., Zuscik, M., Rosier, R.N., Brendan, F., Boyce, B.F., O'Keefe, R.J., Schwarz, E.M., 2005. Lead exposure inhibits fracture healing and is associated with increased chondrogenesis, delay in cartilage mineralization, and a decrease in osteoprogenitor frequency. *Environ. Health Perspect.* 113, 749–755.

Castellanos, P., Mateo, R., Reglero, M.M., Estes, M.C., Fernández-Santos, M.R., Garde, J.J., 2008. In vitro effects of lead on fatty acid composition, oxidative stress biomarkers and quality of ram spermatozoa. *Toxicol. Environ. Chem.* 90, 1163–1175.

Church, M.E., Gwiazda, R., Risebrough, R.W., Sorenson, K., Chamberlain, C.P., Farry, S., Heinrich, W., Rideout, B., Smith, D., 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. *Environ. Sci. Technol.* 40, 6143–6150.

Conover, W.J., 1980. *Practical Nonparametric Statistics*, second ed. John Wiley, New York. 229 pp.

Daszak, P., Cunningham, A.A., Hyatt, A.D., 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop.* 78, 103–116.

Dauwe, T., Bervoets, L., Pinxten, R., Blust, R., Eens, M., 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environ. Pollut.* 124, 429–436.

De Martino, E., 2009. Estudio de home range y estacionalidad en el comportamiento de vuelo de ejemplares de condor andino (*Vultur gryphus*) liberados en la Argentina y Chile. Biology Bachelor Thesis. CAECE University, Buenos Aires, Argentina.

del Hoyo, J., Elliott, A., Sargatal, J., 1994. *Handbook of the Birds of the World*. New World Vultures to Guinea fowl, vol. 2. Lynx Edicions, Barcelona.

Dobson, A., Foufopoulos, J., 2001. Emerging infectious pathogens of wildlife. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 356, 1001–1002.

Donazar, J.A., Travaini, A., Ceballos, O., Rodríguez, A., Delibes, M., Hiraldo, F., 1999. Effects of sex-associated competitive asymmetries on foraging group structure and despotic distribution in Andean condors. *Behav. Ecol. Sociobiol.* 45, 55–65.

Edens, F.W., Garlich, J.D., 1983. Lead-induced egg production decrease in leghorn and japanese quail hens. *Poult. Sci.* 62, 1757–1763.

Espín, S., Martínez-López, E., Jiménez, P., María-Mojica, P., García-Fernández, A.J., 2015. Delta-aminolevulinic acid dehydratase ( $\delta$ ALAD) activity in four free-living bird species exposed to different levels of lead under natural conditions. *Environ. Res.* 137, 185–198.

Ferreira, H., Romano, M., Beldomenico, P., Caselli, A., Correa, A., Uhart, M., 2014. Lead gunshot pellet ingestion and tissue lead levels in wild ducks from Argentine hunting hotspots. *Ecotoxicol. Environ. Saf.* 103, 74–81.

Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. *Proc. Natl. Acad. Sci. U. S. A.* 109, 11449–11454.

Fisher, I.J., Pain, D.J., Thomas, V.J., 2006. Review lead from ammunition sources in terrestrial birds. *Biol. Conserv.* 131, 421–432.

Franson, J.C., Pain, D.J., 2011. Lead in birds. In: Beyer, W.N., Meador, J.P. (Eds.), *Environmental Contaminants in Biota: Interpreting Tissue Concentrations*, second ed. CRC Press, Boca Raton, FL, pp. 563–593.

Friend, M., Franson, J.C., Anderson, W.L., 2009. Biological and societal dimensions of lead poisoning in birds in the USA. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, pp. 34–60.

Glade, A.A., 1988. *Libro Rojo de los vertebrados de Chile*. CONAF, Santiago.

Gangoso, L., Alvarez-Lloret, P., Rodríguez-Navarro, A.A., Mateo, R., Hiraldo, F., Donazar, J.A., 2009. Long-term effects of lead poisoning on bone mineralization in vultures exposed to ammunition sources. *Environ. Pollut.* 157, 569–574.

Gómez-Ramírez, P., Shore, R.F., van den Brink, N.W., van Hattum, B., Bustnes, J.O., Duke, G., Fritsch, C., García-Fernández, A.J., Helander, B.O., Jaspers, V., Krone, O., Martínez-López, E., Mateo, R., Movalli, P., Sonne, C., 2014. An overview of existing raptor contaminant monitoring activities in Europe. *Environ. Int.* 67, 12–21.

Gómez-Ramírez, P., Martínez-López, E., María-Mojica, P., León-Ortega, M., García-Fernández, A.J., 2011. Blood lead levels and ALAD inhibition in nestlings of Eurasian Eagle Owl to assess lead exposure associated to an abandoned mining area. *Ecotoxicology* 20, 131–138.

Grandjean, P., 1976. Possible effect of lead on egg-shell thickness in kestrels 1874–1974. *Bull. Environ. Contam. Toxicol.* 16, 101.

Guillemain, M., Devineau, O., Lebreton, J.D., Mondain-Monval, J.-Y., Johnson, A.R., Simon, G., 2007. Lead shot and teal (*Anas crecca*) in the Camargue, Southern France: effects of embedded and ingested pellets on survival. *Biol. Conserv.* 137, 567–576.

Hendrickson, S.L., Bleiweiss, R., Matheus, J.C., de Matheus, L.S., Jácome, N.L., Pavez, E., 2003. Low genetic variability in the geographically widespread Andean Condor. *Condor* 105, 1–12.

- Hernández, M., Margalida, A., 2009. Assessing the risk of lead exposure for the conservation of the endangered Pyrenean bearded vulture (*Gypaetus barbatus*) population. *Environ. Res.* 109, 837–842.
- Hunt, W.G., Burnham, W., Parish, C.N., Burnham, K.K., Mutch, B., Oaks, J.L., 2006. Bullet fragments in deer remains: implications for lead exposure in avian scavengers. *Wildl. Soc. Bull.* 34, 167–170.
- Jenni, L., Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H., Jenny, D., 2015. The frequency distribution of lead concentration in feathers, blood, bone, kidney and liver of golden eagles *Aquila chrysaetos*: insights into the modes of uptake. *J. Ornithol.* 156, 1095–1103.
- Johnson, C.K., Kelly, T.R., Rideout, B.A., 2013. Lead in ammunition: a persistent threat to health and conservation. *Eco. Health* 10, 455–464.
- Kelly, T.R., Johnson, C.K., 2011. Lead exposure in free-flying Turkey vultures is associated with big game hunting in California. *PLoS One* 6, e15350.
- Kenny, D., Reading, R., Maude, G., Hancock, P., Garbett, B., 2015. Blood lead levels in white-backed vultures (*Gyps africanus*) from Botswana, Africa. *Vulture News* 68, 25–31.
- Lambertucci, S.A., 2010. Size and spatio-temporal variations of the Andean condor *Vultur gryphus* population in north-west Patagonia, Argentina: communal roosts and conservation. *Oryx* 44, 441–447.
- Lambertucci, S.A., Carrete, M., Donazar, J.A., Hiraldo, F., 2012. Large-scale age-dependent skewed sex ratio in a sexually dimorphic avian scavenger. *PLoS One* 7, e46347.
- Lambertucci, S.A., Donazar, J.A., Hiraldo, F., 2010. Poisoning people and wildlife with lead ammunition: time to stop. *Environ. Sci. Technol.* 44, 7759–7760.
- Lambertucci, S.A., Alarcón, P.A., Hiraldo, F., Sánchez-Zapata, J.A., Blanco, G., Donazar, J.A., 2014. Apex scavenger movements call for transboundary conservation policies. *Biol. Conserv.* 170, 145–150.
- Lambertucci, S.A., Donazar, J.A., Delgado Huertas, A., Jiménez, B., Sáez, M., Sánchez-Zapata, J.A., Hiraldo, F., 2011. Widening the problem of lead poisoning to a South American top scavenger: lead concentrations in feathers of wild Andean Condors. *Biol. Conserv.* 144, 1464–1471.
- Lambertucci, S.A., Trejo, A., Di Martino, S., Sánchez-Zapata, J.A., Donazar, J.A., Hiraldo, F., 2009a. Spatial and temporal patterns in the diet of the Andean Condor: ecological replacement of native fauna by exotic species. *Anim. Conserv.* 12, 338–345.
- Lambertucci, S.A., Speziale, K.L., Roggers, T.E., Morales, J.M., 2009b. How do roads affect the habitat use of an assemblage of scavenging raptors? *Biodivers. Conserv.* 18, 2063–2074.
- Lambertucci, S.A., 2007. Biología y conservación del Cóndor Andino (*Vultur gryphus*) en Argentina. *El Hornero* 22, 149–158.
- Locke, L.N., Bagley, G.E., Frickie, D.N., Young, L.T., 1969. Lead poisoning and aspergillosis in an Andean condor. *J. Am. Veterinary Med. Assoc.* 155, 1052–1056.
- López-Lanús, B., Grilli, P., Coconier, E., Di Giacomo, A., Banchs, R., 2008. Categorización de las aves de la Argentina según su estado de conservación. Informe de Aves Argentinas/AOP y Secretaría de Ambiente y Desarrollo Sustentable, Buenos Aires, Argentina.
- Madry, M.M., Kraemer, T., Kupper, J., Naegeli, H., Jenny, H., Jenni, L., Jenny, D., 2015. Excessive lead burden among golden eagles in the Swiss Alps. *Environ. Res. Lett.* 10, 034003.
- Martínez-López, E., Martínez, J.E., María-Mojica, P., Penalver, J., Pulido, M., Calvo, J.F., García-Fernández, A.J., 2004. Lead in feathers and  $\delta$ -aminolevulinic acid dehydratase activity in three raptor species from an unpolluted Mediterranean forest (Southeastern Spain). *Archiv. Environ. Contam. Toxicol.* 47, 270–275.
- McCullagh, P., Nelder, J.A., 1989. *Generalized Linear Models*, vol. 37. CRC press.
- Nam, D.H., Lee, D.P., 2009. Abnormal lead exposure in globally threatened Cinereous vultures (*Aegypius monachus*) wintering in South Korea. *Ecotoxicology* 18, 225–229.
- Ogada, D.L., Keesing, F., Virani, M.Z., 2012. Dropping dead: causes and consequences of vulture population declines worldwide. *Ann. N. Y. Acad. Sci.* 1249, 57–71.
- Ogada, D., Shaw, P., Beyers, R.L., Buij, R., Murn, C., Thiollay, J.M., Beale, C.M., Holdo, R.M., Pomeroy, D., Baker, N., Kruger, S.C., Botha, A., Virani, M.Z., Monadjem, A., Sinclair, A.R.E., 2015. Another continental vulture crisis: Africa's vultures collapsing toward extinction. *Conserv. Lett.* 9, 89–97.
- Ortega, A., Salvador, M.F., Rodríguez, P., Saldoval, T., 2010. Informe Final de Investigación, Proyecto de conservación in-situ y ex-situ del cóndor andino en Ecuador. Fundación Zoológica de Ecuador.
- Pain, D.J., Meharg, A.A., Ferrer, M., Taggart, M., Penteriani, V., 2005. Lead concentrations in bones and feathers of the globally threatened Spanish imperial eagle. *Biol. Conserv.* 121, 603–610.
- Pain, D.J., Fisher, I.J., Thomas, V.G., 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, USA. <http://dx.doi.org/10.4080/ilsa.2009.0108>.
- Parish, C.N., Hunt, W.G., Feltes, E., Sieg, R., Orr, K., 2009. Lead exposure among a reintroduced population of California condors in northern Arizona and southern Utah. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, ID, pp. 259–264. Available. <http://www.peregrinefund.org/subsites/conference-lead/PDF/0217%20Parish.pdf> [accessed 2 Apr 2016].
- Pattee, O.H., 1984. Eggshell thickness and reproduction in American kestrels exposed to chronic dietary lead. *Archiv. Environ. Contam. Toxicol.* 13, 29–34.
- Pattee, O.H., Carpenter, J.W., Fritts, S.H., Rattner, B.A., Wiemeyer, S.N., Royle, J.A., Smith, M.R., 2006. Lead poisoning in captive Andean condors (*Vultur gryphus*). *J. Wildl. Dis.* 42, 772–779.
- Ramos, V.A., 1999. Plata tectonic setting of the Andean Cordillera. *Episodes* 22, 183–190.
- Saggese, M.D., Quaglia, A., Lambertucci, S.A., Bó, M.S., Sarasola, J.H., Pereyra-Lobos, R., Maceda, J.J., 2009. Survey of lead toxicosis in free-ranging raptors from central Argentina. In: Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G. (Eds.), *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, pp. 223–231.
- Shah, F., Kazi, T.G., Afridi, H.I., Baig, J.A., Khan, S., Kolachi, N.F., Wadhwa, S.K., Shah, A.Q., 2010. Environmental exposure of lead and iron deficit anemia in children age ranged 1–5years: a cross sectional study. *Sci. Total Environ.* 408, 5325–5330.
- Speziale, K.L., Lambertucci, S.A., Olsson, O., 2008. Disturbance from roads negatively affects Andean Condor habitat use. *Biol. Conserv.* 141, 1765–1772.
- Snoeijs, T., Dauwe, T., Pinxten, R., Vandesande, F., Eens, M., 2004. Heavy metal exposure affects the humoral immune response in a free-living small songbird, the great tit (*Parus major*). *Archiv. Environ. Contam. Toxicol.* 46, 399–404.
- Taylor, T.G., 1970. The role of the skeleton in eggshell formation. *Ann. Biol. Anim. Biochim. Biophys.* 10, 83–91.
- Watson, R.T., Fuller, M., Pokras, M., Hunt, W.G., 2009. *Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans*. The Peregrine Fund, Boise, Idaho, USA.
- Williams, R.S., Jara, J.L., Matsufuji, D., Plenge, A., 2011. Trade in Andean condor *Vulture gryphus* feathers and body parts in the city of Cusco and the Sacred Valley, Cusco region. Peru. *Vulture News* 61, 16–26.