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SANTA CRUZ

LEAD EXPOSURE AND HORMONAL STRESS RESPONSE IN CALIFORNIA CONDORS

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ZEKA E. GLUCS

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The Dissertation of

Zeka E. Glucs is approved:

Professor Donald R. Smith, Chair

Associate Adj. Professor, Myra E. Finkelstein

Christopher Tubbs, Ph.D.

Professor Karen Otteman

Tyrus Miller, Vice Provost and Dean of Graduate Studies

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LEAD EXPOSURE AND HORMONAL STRESS RESPONSE IN CALIFORNIA CONDORS

Zeka Elaine Glucs

ABSTRACT

The primary factor inhibiting the recovery of the critically endangered California condor (*Gymnogyps californianus*) is lead poisoning from ingestion of spent lead ammunition. My dissertation research documents the sources and effects of lead poisoning in condors, and provides the first information on the effects of lead on the hormonal stress response in condors. My first chapter aims to help identify sources of lead to condors by investigating three illegal condor shooting events. I use lead isotope ratios of condor tissues as well as ingested and embedded ammunition to find probable cause to link these shooting events. For my second and third chapters, I provide what are to the best of my knowledge the first data on the hormonal stress response in condors, and how lead exposure impacts this stress response. We know the vast majority of wild California condors are frequently lead poisoned, but we have limited data on how these frequent lead poisoning events affect the birds' physiology. Lead poisoning has been shown in other organisms to heighten the hormonal stress response, which can lead to suppressed fitness in wild birds. My findings indicate that this dysfunction is occurring in wild condors, as I found a positive association between hormonal stress response outcomes and the amount of time a condor spends at risk for lead poisoning (foraging outside the management area). Interestingly, I also found that the annual frequency of a condor feeding on marine mammals, which contain high levels of hormone-disrupting chemicals such as

polychlorinated biphenyls, is also associated with hormonal stress response elevation. My work fills a critical lack in our understanding of how long-term contaminant exposure might impact the California condor recovery effort, and has important implications for other scavenging species exposed to lead and other environmental contaminants worldwide. Future study is needed to investigate whether the altered hormonal stress response is impairing the fitness, survival and reproduction of wild condors.

This dissertation is dedicated to pre-eminent biologist and friend, Michael Tyner, who dedicated his life to condor conservation, and is almost certainly flying with them now. His encouragement to pursue a graduate thesis on lead poisoning in California condors propelled me onto this path. Thank you Mike.

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The text of this dissertation includes reprints of the following previously published material:

Finkelstein, M. E., Kuspa, Z. E., Welch, A., Eng, C., Clark, M., Burnett, J., Smith, D. R. 2014. Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis. Environmental Research 134C, 270–279.

CHAPTER 1: INTRODUCTION

Lead poisoning has been a human health problem for centuries (Pokras and Kneeland, 2008). Exposure to lead can result in serious neurological damage (Lucchini et al., 2012; Sanders et al., 2009; White et al., 2007), kidney failure (Sabolić, 2006), heart disease (Lanphear et al., 2018), and is implicated as a reproductive toxin (Benoff et al., 2000; Hernández-Ochoa et al., 2005; Telisman et al., 2007). To protect human health, lead has been removed from gasoline and paint in many countries. However, lead-based ammunition remains a substantial, largely unregulated source of lead knowingly discharged into the environment and poses significant health risk for both humans and wildlife (Arnemo et al., 2016; Bellinger et al., 2013).

Lead-based ammunition ingestion in California condors

Lead poisoning from the ingestion of spent lead-based ammunition is the primary mortality factor for adult California condors (Rideout et al., 2012) and is affecting the species on a population level, preventing their recovery in the wild (Finkelstein et al., 2012). Their specialized diet and feeding behaviors elevate their risk of lead ammunition ingestion and resulting lead exposures.

The California condor is an obligate scavenger which feeds predominantly on large ungulate and marine mammal carrion (Koford, 1953; Snyder and Snyder, 2000). Condors will also readily ingest smaller carcasses (e.g., rodents). Since their sense of smell is not as highly developed as other new world vultures (turkey vulture; greater yellow-headed vulture, *Cathartes melambrotus*; and lesser yellow-headed vulture, *Cathartes burrovianus*) condors are believed to forage by sight, finding food

by visually locating larger carcasses, or large aggregations of carcasses, on their own or by following other scavengers to their meals (Snyder and Snyder, 2000). Hunting is a major cause of mortality for large and medium sized land mammals (≈30% of mortalities, Collins and Kays 2011). In addition, large-scale small mammal hunting such as ground squirrel depredation, where >100 individuals can be shot per day, have the potential to poison avian scavengers (Herring et al., 2016). The condor's penchant for highly visible or numerous carcasses may increase their risk of encountering game killed by humans with lead-based ammunition.

Lead bioavailability and absorption in avian species

The avian digestive tract can vary between species in three basic ways to achieve optimal efficiency: 1) degree of mechanical digestion (strength of gizzard, use of grit), 2) length of intestine, and 3) pH of gastric fluid. Avian scavengers do not use grit to aid in digestion. The differences in intestine length among North American vultures, eagles, and condors, are not available in print but there are some published data on differences in pH in gastric fluid in ecologically similar species.

In birds, hydrochloric acid is secreted by the proventriculous, the avian equivalent to the mammalian stomach. The acidity of digestive secretions vary between species and appear to be linked to both diet and foraging strategy (Denbow et al., 2000). For example, low pH stomachs (basal pH 1.35 ± 0.14) allow wandering albatross (*Diomedea exulans*) to ingest large amounts of food at one time, and digest it quickly so they can forage over long distances for patchy resources (Grémillet et al., 2012). Old world vultures (e.g.. white-backed griffon vulture, *Gyps africanus*) have also been documented as having highly acidic stomachs as well

(digesting pH 1.23 ± 0.25, Houston and Cooper 1975). The pH of the California condor digestive tract is not known, but one would predict that it would be similar to old world vultures based on its convergent natural history. A high pH in the GI tract may make these species more susceptible to lead poisoning via ingestion since acidic aqueous solutions (particularly hydrochloric acid) more readily dissolve lead (Agency for Toxic Substances and Disease Registry, 2007).

Lead crosses the intestinal membrane through calcium pathways in its Pb^{2+} form, but can be transported to the lumenal surface of the intestine complexed with other molecules in the more neutral environment (Oomen et al., 2003). Lead can also compete with calcium for active sites of intestinal binding proteins (Fullmers and Edelsteinp, 1985). In fact, diets high in calcium (or more specifically calcite) have been shown to be protective against lead absorption in birds, potentially by increasing gizzard pH, increasing carbonate concentrations and promoting lead precipitation in the intestine, or increasing competition for intestinal absorption (Martinez-Haro et al., 2009).

Toxic effects of lead in wildlife

Wildlife ingest lead in the form of manufactured products including lead-based ammunition, historic paint, and fishing weights (Fisher et al. 2006), which can induce lead toxicosis, a deadly form of lead poisoning characterized by paralysis, seizures, and ultimately organ failure (Aguilar et al., 2012) as well as sub-lethal effects, which are described below (e.g. Ecke et al., 2017; Ferreyra et al., 2015). Lead related deaths have been documented in wild birds since the turn of the

century (see Table 1), and some place the birth of wildlife toxicology at the discovery of avian lead poisoning (Rattner, 2009).

Table 1. Select Publications Documenting Avian Mortalities from Lead Toxicosis

Species	Type of Study	Authors	Year
Pheasant (*Phaesianus colchicus*)	Observational	Calvert	1876
Waterfowl	Observational	Grinell	1894
Domestic chicken (*Gallus domesticus*)	Experimental	Thomas and Shealy	1932
Canada goose (*Branta canadensis*)	Experimental	Cook and Trainer	1966
California condor (*Gymnogyps californianus*)	Observational	Janssen et al.	1986
Turkey vulture (*Cathartes aura*)	Experimental	Carpenter et al.	2003

While lead-related deaths can be quantified, the sub-lethal effects of chronic lead exposure are more difficult to assess in wildlife, including avian scavengers (Hunt, 2012). Exposure to lead can affect the hypothalamic-pituitary-adrenal (HPA) axis, the part of the endocrine system responsible for modulating the release of glucocorticoid hormones in response to stress (e.g., Virgolini et al. 2006; Gump et al. 2008; Rossi-George et al. 2009; Fortin et al. 2012). Alterations to this system can affect survival and reproductive success in wild animals, both indirectly and directly (Breuner, 2011; Whirledge and Cidlowski, 2010). Glucocorticoids induce metabolic and behavioral changes important for meeting energy demands and avoiding dangerous situations, and when these hormones are present in inappropriate concentrations behavioral maladjustments (Davies et al., 2007) and serious disease can develop (e.g. Cushing's Disease; Guyton and Hall, 2006).

To date, only three associative studies have been conducted on lead and the HPA axis in wild birds. Baos et al. (2006) found a significant positive relationship between blood lead levels and maximum glucocorticoid release in response to stress in white stork (*Ciconia ciconia*) chicks. Meillère et al. (2016) observed a significant positive correlation between lead and other metals and feather corticosterone concentrations in juvenile and adult common blackbirds (*Turdus merula*). Finally, Provencher et al. (2016) measured blood lead and baseline corticosterone concentrations in wild common eiders (*Somateria mollissima*) along with metrics of body condition and reproductive fitness and found no relationship between blood lead (<5 µg/dL) and baseline corticosterone, but a strong negative relationship between blood lead and body condition upon arrival to breeding site. Overall, these studies suggest that lead exposure may increase stress-induced glucocorticoid release, but effects on baseline glucocorticoid levels in birds are not yet evident.

Comparative analysis of stress endocrinology

The highly conserved vertebrate HPA axis

The stimulation of the HPA axis cascade, endocrine glands, and hormones released are remarkably comparable between birds and mammals and highlight the importance of the HPA axis to survival and fitness in vertebrates over evolutionary history (Wingfield et al., 1999). The HPA axis response differs from the immediate release of adrenaline and noradrenaline in response to a threat, often referred to as the “fight or flight response” (Guyton and Hall, 2006). Instead, the HPA axis culminates in the release of steroid hormones which have both longer time to action (5-10 minutes to increase, hours to maximize) and longer residence time in the blood

($t_{1/2}$≈40 min), the HPA axis response is more suited for long-term stress and recovery such as limited food availability or inclement weather.

In response to a stressor (e.g., energy deficiency, exercise, inclement weather, intraspecific conflict, physical restraint) a hormone cascade is initiated by neurons in the hypothalamus and culminates with the release of glucocorticoids into the bloodstream by the adrenal gland (Harvey et al., 1984, summarized in Figure 1). Glucocorticoids are non-polar and partition with plasma proteins. 85-99% of glucocorticoid circulates bound to corticosteroid binding globulin (a.k.a transcortin), which resists metabolism and increases glucocorticoid residence time in the blood (Carsia, 2015).

Glucocorticoids act on target cells by diffusing through the cell membrane to bind with cytosolic receptors. Hormone-receptor complexes translocate to the nucleus and form a hormone-receptor dimer complex bound to a hormone response element on gene promoters upstream of a target gene, recruiting co-activators and facilitating the transcription of the target gene (Carsia, 2015). The genetic mechanism of action creates an additional time lag (~60-90 minutes) between stimulation of the HPA axis cascade and the effects on target cells of glucocorticoids. There is some evidence for a non-genomic pathway of action for glucocorticoids in which hormones activate receptors in the cell membrane coupled to second messenger systems that elicit effects on the nervous system and cardio-pulmonary system in a matter of minutes (Stahn and Buttgereit, 2008).

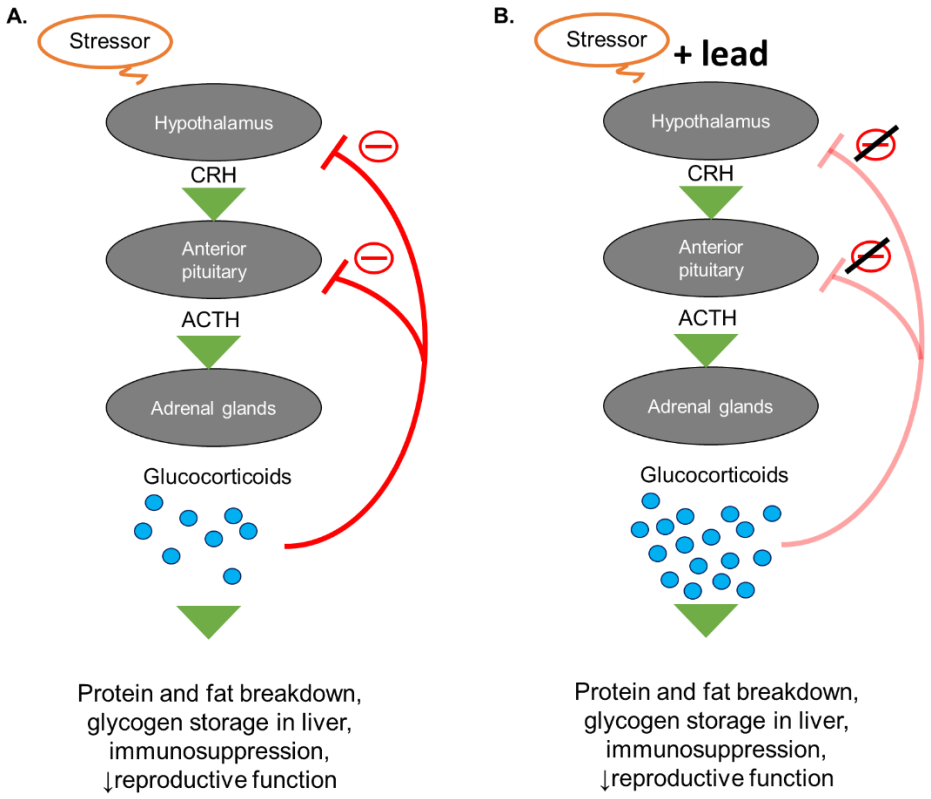

Figure 1. Diagram of HPA axis cascade and lead-induced dysfunction: (A) The hypothalamus releases the peptide corticotropin-releasing hormone (CRH). Receptors on corticotropic cells within the anterior pituitary gland are activated by CRH to release adrenocorticotropin (ACTH) from precursor peptide proopiomelanocortin. ACTH acts on cells in the adrenal cortex to stimulate release of the glucocorticoid hormones cortisol (primary stress hormone in humans), corticosterone (primary stress hormone in birds), and other steroid hormones of limited affinity for glucocorticoid receptors in target cells. (B) Illustration of the proposed mechanism for lead exposure mediated HPA axis dysfunction (Rossi-George et al., 2009). Lead exposure increases HPA axis responsiveness by impairing the negative feedback of glucocorticoids on the neurons in the hypothalamus and/or receptors on the anterior pituitary

Glucocorticoids are synthesized from cholesterol primarily in the zona fasciculata of the adrenal cortex, but also in other organs such as the brain (Guyton and Hall, 2006; Jung-Testas and Baulieu, 1998). In vertebrates the most biologically relevant glucocorticoid hormones are cortisol and corticosterone (Figure 2) and the particular glucocorticoid that is primarily released by the adrenals differs between taxa (*Bentley, 1998* in Sheriff et al. 2011). Corticosterone is the dominant glucocorticoid in the avian stress response, while cortisol is dominant in humans and many other mammals (Palme et al., 2005). Some species (e.g., bighorn sheep, *Ovis canadensis*) secrete both cortisol and corticosterone and these cases the two glucocorticoids can have different targets (Koren et al., 2012b).

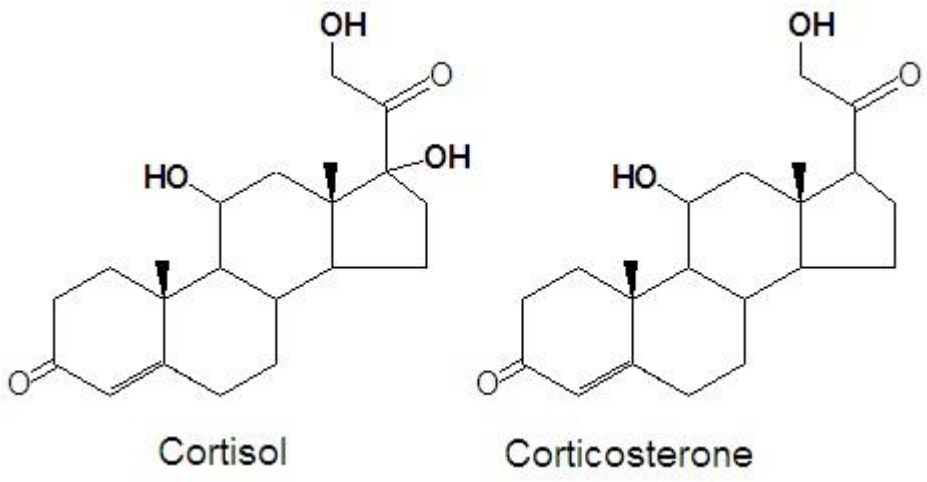

Figure 2. Molecular structure of primary glucocorticoids. Adapted from: http://en.citizendium.org/wiki/steroid

Glucocorticoids have a robust effect on intermediary metabolism, increasing blood glucose at the expense of protein and fat storage while storing energy as glycogen for future need (Guyton and Hall, 2006). Glucocorticoid hormones also play an important role in immunosuppression. When leukocytes are activated and release

pro-inflammatory cytokines, glucocorticoids are subsequently released to counter the immune system response by decreasing vasodilation, capillary permeability, lymphocyte production, lymphocyte migration, and damage from lysosomes (Guyton and Hall, 2006). While this effect may be maladaptive in times of illness, suppressing inflammation can be beneficial during times of stress. Unchecked by glucocorticoids, an inflammatory response can cause significant tissue damage and pain (Guyton and Hall, 2006).

Metabolism of glucocorticoids

As mentioned previously, glucocorticoids circulate bound to corticoid binding globulins. While bound glucocorticoids are protected from enzyme action, free glucocorticoids are metabolized by the liver through conjugation (sulfonation or glucuronidation) or by cytochrome P450s, which inactivate the hormone and increase their water solubility for excretion via urine or feces (Möstl et al., 2005). In mammals, the primary excretion route varies among species, and in birds feces and urates are mixed so primary excretion route is unclear (Palme et al., 2005). These metabolites can be measured in urine and fecal material using either radio-immunoassay (RIA), enzyme immunoassay (EIA), or LCMS.

In studies that used high performance liquid chromatography to separate fecal glucocorticoid metabolites most of the immunoreactivity was associated with highly polar molecules (Hirschenhauser et al., 2012; Lèche et al., 2011; Staley et al., 2007). After defecation bacterial enzymes continue to metabolize glucocorticoid metabolites, which necessitates analysis or freezing of fecal samples within 15 minutes if possible (Herring and Gawlik, 2009; Möstl et al., 2005). Freezing fecal

samples at -20˚C can allow for increases in immunoreactive compounds over time (Khan et al., 2002), but when frozen and stored at -80˚C, avian fecal samples have been found to be relatively stable (Herring and Gawlik, 2009).

Described avian stress responses

Glucocorticoids have been measured in tissues of several bird species, but the full glucocorticoid stress response (baseline, peak, and return to baseline) has been described in only a small portion of studies (Table 2). For all bird species studied, corticosterone appears to be the primary glucocorticoid. That being said, at least one species (e.g., house sparrows, *Passer domesticus*) has detectable levels of cortisol in their feathers and plasma, however the corticosterone:cortisol ratio is approximately 50.6 in plasma (Koren et al., 2012a). Interestingly, concentrations of the two hormones were significantly correlated in feathers when they both were detected (correlation by randomizations: $r = 0.40$, $n = 50$, $p < 0.01$), and more feathers had detectable amounts of cortisol (41%) than corticosterone (33%). Developing birds will concentrate cortisol in immune tissues (Schmidt and Soma, 2008). These observations could indicate peripheral synthesis of cortisol in feather and immune tissues rather than adrenal cortisol synthesis.

The variation in HPA axis stress response magnitude and duration between species and among individuals is substantial (Sheriff et al., 2011; Wilcoxen et al., 2011). In studies that measured glucocorticoid metabolites in avian feces (feces and urates mixed), a biphasic response to stressors was sometimes observed (Hirschenhauser et al., 2012; Staley et al., 2007). The biphasic excretion pattern most likely results from different excretion times for fecal and urate pathways

Table 2. Published glucocorticoid measurements in avian species

Species	E/O	Stressor	Tissue Sample	Baseline CORT (ng/g feces, ng/g feather, ng/mL plasma)	Peak CORT (ng/g feces, ng/mL plasma)	Time (Stressor to Peak)	Time (Peak to Recovery)	Analysis Technique	Authors	Year
White Stork (*Ciconia ciconia*)	O	Capture-Restraint	plasma	~7-40	~32-70	NA	NA	RIA/HPLC	Baos et al.	2006
Golden Eagle (*Aquila chrysaetos)*	E	Saline Injection	feces	2.22 ± 0.16	2.46 ± 0.16	8 hrs	NA	RIA/HPLC	Staley et al.	2007
Peregrine Falcon (*Falco peregrinus*)	E	Saline Injection	plasma	1.23 ± 0.13	NA	NA	NA	RIA/HPLC	Staley et al.	2007
Barred Owl (*Strix varia*)	E	Injection	feces	NA	NA	3 hrs	NA	RIA	Wasser and Hunt	2005
California Spotted Owl (*Strix occidentalis occidentalis)*	O	NA	feces	80.1	NA	NA	NA	RIA	Tempel and Gutierrez	2003, 2004
Great Horned Owl (*Bubo virginianus*)	E	Injection	feces	NA	NA	3 hrs, 10.75 hrs	NA	RIA/HPLC	Wasser and Hunt	2005
Northern Spotted Owl (*Strix occidentalis caurina*)	O	NA	feces	nestlings: 60-120, adults: 25-100	NA	NA	NA	RIA/HPLC	Wasser and Hunt	2005
Greater Rhea (*Rhea americana*)	E	Saline Injection	feces	15.79 ± 2.3	73.2 ± 14.3	4-6 hrs	2 hrs	RIA	Lèche et al.	2011
Greater Rhea (*Rhea americana*)	E	Saline Injection	plasma	7.3 ± 1.3	102 ± 12.9	30 min	24 hrs	RIA	Lèche et al.	2011
Domestic Chicken (*Gallus domesticus*)	E	Hormone Injection	feces	NA	NA	1-2 hrs, 2-4 hrs	NA	HPLC/RIA	Hirschenhauser et al.	2012
Japanese Quail (*Coturnix japonica)*	E	ACTH challenge, CORT injection	feces	NA	NA	45-55 min, 3-3.5 hrs	NA	HPLC/RIA	Hirschenhauser et al.	2012
European Starlings (*Sturnus vulgaris*)	E	ACTH challenge, CORT injection	plasma	~6 ± 1	NA	NA	NA	RIA	Cyr et al.	2007
European Stonechat (*Saxicola rubicola*)	E	NA	plasma	NA	NA	Male:3.7 hrs, Female:24 hrs	NA	RIA	Canoine et al.	2002
Great Tits (*Parus major*)	O	Capture-Restraint	plasma	Male: 14.2 ± 2.4, Female: 13.5 ± 2.7	Male: ~11-65	NA	NA	EIA	Ouyang et al.	2012
House Sparrow (*Passer domesticus*)	O	NA	feather	4.1-372.9	NA	NA	NA	LC/MS-MS	Koren et al.	2012

(Möstl et al., 2005; Sheriff et al., 2011). Urate excretion of glucocorticoid metabolites is faster than that of fecal excretion (Möstl et al., 2005; Staley et al., 2007).

Stress-fitness tradeoffs

Proper stress response to labile perturbation factors increases survival

Glucocorticoid release is a mechanism for regulating energy homeostasis in wild organisms. Energy demands are additive and they fluctuate just as availability of energy sources change over time in amount and spatial density (Bridge et al., 2009; McEwen and Wingfield, 2003). Energy demands can be increased by inclement weather, illness, or increased foraging effort. Stressors can also increase energy demand (e.g., social subordination, predation pressure). Exceeding the energy "threshold" leads to an HPA axis response which enables the individual to either endure or escape a stressful situation (Wingfield et al., 1999). Psychological stress can also trigger an HPA axis response, perhaps because of an anticipated heightened energy need. For example, decreased predictability of food availability was associated with higher blood corticosterone concentration in Florida scrub jays (*Aphelocoma coerulescens*) (Bridge et al., 2009). Furthermore, just the sight of food after fasting reduced corticosterone levels in domestic fowl (Harvey et al., 1984). Whether an individual withstands a stressful situation in place or escapes to a temporary haven, glucocorticoid release mobilizes the energy stores needed to overcome the stressful situation with minimum adverse effect to organism health and fitness.

If the stressful situation requires too much energy (HPA axis activated for too long), life history stages, such as reproduction, may be suspended to prioritize

immediate survival. While this would certainly reduce reproductive output for that breeding season, reducing short-term reproductive success can increase long-term fitness. Especially in long-lived animals, foregoing one breeding season for the sake of survival allows for many more future breeding attempts. Theoretically, longer lived species should have a lower threshold for triggering emergency life history stages than short-lived species (Wingfield et al., 1999).

Consequences of increased magnitude of glucocorticoid response in avian species

Glucocorticoids do not always show a linear relationship with survival or reproductive success in wild birds (Busch and Hayward, 2009), but several studies have documented associations between glucocorticoid elevation and adverse effects on fitness. Stress-induced glucocorticoid levels have been found to be negatively associated with chance of survival and recruitment as breeders (Blas et al., 2007) and chick growth rates (Albano et al., 2015), and positively associated with nest abandonment (Love et al., 2004; Ouyang et al., 2012). Longer handling durations lead to later onset of breeding and higher probabilities of death by avian cholera in breeding female common eiders (Buttler et al., 2011).

Consequences of chronically elevated glucocorticoids

Actions of glucocorticoids, while essential in a short term stress response (hours to days), become maladaptive if in place for longer durations (weeks to months). Under conditions of chronic stress that produce chronically elevated circulating glucocorticoid levels, excessive catabolism of fat and protein stores for energy can weaken the individual and keep them in emergency life history stages for a longer periods of time (Guyton and Hall, 2006; Wingfield and Kitaysky, 2002).

Chronic stress can both directly and indirectly cause reproductive failure. As mentioned, loss of energy stores may preclude reproduction for that breeding season. Gonad functions preceding conception are very sensitive to energy availability, especially in females (Ellison, 2003). Glucocorticoid release in response to energy deficits can also directly decrease reproductive success through stimulation of the gonadotropin-inhibitory hormone (Iwasa et al., 2017), which ultimately suppresses the release of reproductive hormones such as testosterone (Deviche et al., 2012). In red crossbills (*Loxia curvirostra*), opportunistic breeders that live in harsh environments, winter breeders show suppressed corticosterone release compared to summer breeders, potentially to combat the negative effects of glucocorticoids on reproductive success (Cornelius et al., 2012).

Glucocorticoid-mediated immunosuppression (Martin, 2009), can affect survival in individuals exposed to an infectious diseases such as West Nile Virus (Jankowski et al., 2010; Owen et al., 2012). While virtually all wild California condors are vaccinated for West Nile Virus, increased susceptibility to other infectious avian diseases (e.g., avian influenza) could have serious repercussions for a vulnerable population. However, not all immune response may be affected by corticosterone and each species may have different reactions to glucocorticoids (Cyr and Michael Romero, 2007).

Consequences of insufficient glucocorticoid release

Variations in HPA axis reactivity to stress (peak glucocorticoid – basal glucocorticoid) have been associated with behavioral differences in humans. Children with lower corticosterone reactivity to a simulated parental conflict are more

likely to exhibit externalizing symptoms (e.g., angry outbursts, law-breaking, hyperactivity) in the future (Davies et al., 2007). The consequences of subtle behavioral changes would be difficult to assess in a free-living population of wild birds, but may play a role in important factors for reproduction and survival such as social status.

Since the proper stress response is evidently necessary for survival of environmental perturbation factors (Wingfield et al., 1999), an individual with a suppressed glucocorticoid response may be less equipped to survive times of increased metabolic or psychological stress. Inadequate suppression of inflammation would also be a concern for individuals with low glucocorticoids. The consequences of low glucocorticoids in wildlife are not often documented (Herring et al., 2012).

Effects of lead on vertebrate stress physiology

Effects of early life exposure to lead on HPA axis

Lead exposure *in utero* (>5 µg Pb/dL blood) and in early childhood (<15 µg Pb/dL blood) have been associated with heightened salivary cortisol response to stress in children and increased duration of glucocorticoid elevation (Gump et al., 2008). In the single study on early-life lead exposure and avian adrenal activity, blood lead concentrations were positively correlated with maximum plasma corticosterone concentrations in white stork chicks during a capture-restraint stress test (Baos et al., 2006). The increased magnitude and duration of glucocorticoid release indicates inhibition of the negative feedback mechanism of the HPA axis. This can be mediated by down-regulation of intracellular receptors in parvocellular neurons in response to lead exposure (Rossi-George et al., 2009) and to other

environmental factors (infection, Shanks and Meaney 1995; handling, Meaney et al. 1996).

Effects of chronic lead exposure on adult HPA axis

Fortin et al. (2012) measured ACTH and cortisol in occupational exposed adult men with blood lead concentrations up to 31 µg/dL. Blood lead was negatively associated with baseline cortisol levels. The magnitude of the cortisol response compared to that of adrenocorticotropic hormone (ACTH) release also was suppressed in participants with higher blood leads. This suggested a dysfunction of the HPA axis cascade at the ACTH receptor level in adrenal cortex cells. To date, no study has measured the effect of frequent, episodic, and acute lead exposure on adult avian adrenocortical activity. The mechanism of lead-induced dysfunction of the HPA axis is also unknown. Since stress itself can induce similar effects to lead exposure, and even exacerbate lead exposure effects, both variables must be controlled for.

Research Questions and Broader Impacts

Research Questions

My research explores several significant knowledge gaps regarding lead exposure and the stress response in California condors. In my second chapter, I investigate three illegal shooting events of California condors. By comparing lead isotope ratios in condor tissues and associated fragments found in their bodies, I was able to connect the shooting events to a similar source and time frame, and contrast embedded vs. ingested lead-based ammunition as sources of lead in condors. In my third chapter, I test the accuracy and precision of a glucocorticoid hormone

measurement technique for California condor plasma, urates, and feather and examine the glucocorticoid levels wild and captive individuals following capture and restraint. In my fourth chapter I explore whether lead exposure and other factors are altering the glucocorticoid response in condors. I compare glucocorticoid levels between chronically lead-exposed wild condors, and captive non-lead-exposed condors. Within the wild population I employ a USFWS-NPS-UCSC database on lead exposure histories, movement data, and feeding information for each bird to identify connections between sub-lethal exposures to environmental contaminants and HPA axis response to stress.

Broader Impacts

California condors have become the poster child for wildlife poisoning from lead-based ammunition in the United States, but they are far from the only scavenger impacted by its use. Knowledge gained from studying the sources of lead and the effects of lead exposure in the condor will continue inform our understanding of lead exposure in avian scavengers worldwide. And finally, my work highlights the potential pitfalls and challenges for researchers aiming to measure effects on glucocorticoid release in a previously unstudied, free-ranging species.

References

Agency for Toxic Substances and Disease Registry, 2007. ToxGuide for Lead (Pb).

Aguilar, R.F., Yoshicedo, J.N., Parish, C.N., 2012. Ingluviotomy tube placement for lead-induced crop stasis in the California condor (*Gymnogyps californianus*). J. Avian Med. Surg. 26, 176–81.

Albano, N., Santiago-Quesada, F., Masero, J.A., Sánchez-Guzmán, J.M., Möstl, E., 2015. Immunoreactive cortisone in droppings reflect stress levels, diet and growth rate of gull-billed tern chicks. Gen. Comp. Endocrinol. 213, 74–80. doi:10.1016/j.ygcen.2015.02.019

Arnemo, J.M., Andersen, O., Stokke, S., Thomas, V.G., Krone, O., Pain, D.J., Mateo, R., 2016. Health and environmental risks from lead-based ammunition: science versus socio-politics. Ecohealth 13, 618–622. doi:10.1007/s10393-016-1177-x

Arnold, T.W., 2010. Uninformative Parameters and Model Selection Using Akaike's Information Criterion. J. Wildl. Manage. 74, 1175–1178. doi:10.2193/2009-367

Bakker, V.J., Smith, D.R., Copeland, H., Brandt, J., Wolstenholme, R., Burnett, J., Kirkland, S., Finkelstein, M.E., 2016. Effects of lead exposure, flock behavior, and management actions on the survival of California condors (*Gymnogyps californianus*). Ecohealth 1–14. doi:10.1007/s10393-015-1096-2

Baos, R., Blas, J., Bortolotti, G.R., Marchant, T. a., Hiraldo, F., 2006. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (*Ciconia ciconia*) exposed to heavy metal and arsenic contamination. Environ. Health Perspect. 114, 1497–1501. doi:10.1289/ehp.9099

Baugh, A.T., Oers, K. van, Dingemanse, N.J., Hau, M., 2014. Baseline and stress-induced glucocorticoid concentrations are not repeatable but covary within individual great tits (*Parus major*). Gen. Comp. Endocrinol. 208, 154–163. doi:10.1016/j.ygcen.2014.08.014

Bellinger, D.C., Burger, J., Cade, T.J., Cory-Slechta, D.A., Finkelstein, M., Hu, H., Kosnett, M., Landrigan, P.J., Lanphear, B., Pokras, M.A., Redig, P.T., Rideout, B.A., Silbergeld, E., Wright, R., Smith, D.R., 2013. Health risks from lead-based ammunition in the environment. Environ. Health Perspect. 121. doi:10.1289/ehp.1306945

Benoff, S., Jacob, A., Hurley, I.R., 2000. Male infertility and environmental exposure to lead and cadmium. Hum. Reprod. Update 6, 107–21.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. Proc. Natl. Acad. Sci. U. S. A. 104, 8880–8884.

Bortolotti, G.R., Marchant, T. a., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct. Ecol. 22, 494–500. doi:10.1111/j.1365-2435.2008.01387.x

Bortolotti, G.R., Marchant, T., Blas, J., Cabezas, S., 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. J. Exp. Biol. 212, 1477–82. doi:10.1242/jeb.022152

Braun, E.J., 2014. Osmoregulatory Systems of Birds, Sturkie's Avian Physiology: Sixth Edition. Elsevier. doi:10.1016/B978-0-12-407160-5.00012-9

Breuner, C., 2011. Stress and reproduction in birds. Horm. Reprod. Vertebr. 129–151.

Breuner, C.W., Orchinik, M., 2009. Pharmacological characterization of intracellular, membrane, and plasma binding sites for corticosterone in house sparrows. Gen. Comp. Endocrinol. 163, 214–224. doi:10.1016/j.ygcen.2009.01.027

Bridge, E.S., Schoech, S.J., Bowman, R., Wingfield, J.C., 2009. Temporal predictability in food availability: effects upon the reproductive axis in Scrub-Jays. J. Exp. Zool. A. Ecol. Genet. Physiol. 311, 35–44. doi:10.1002/jez.493

Burnett, L.J., Sorenson, K.J., Brandt, J., Sandhaus, E.A., Ciani, D., Clark, M., David, C., Theule, J., Kasielke, S., Risebrough, R.W., 2013. Eggshell thinning and depressed hatching success of California condors reintroduced to central California. Condor 115, 477–491. doi:10.1525/cond.2013.110150

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Interference: A Practical Information-Theoretic Approach, 2nd ed. Springer-Verlag, New York.

Busch, D.S., 2010. Measuring stress in conservation settings: A reply to Linklater. Biol. Conserv. 143, 1039–1040. doi:10.1016/j.biocon.2009.12.028

Busch, D.S., Hayward, L.S., 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol. Conserv. 142, 2844–2853. doi:10.1016/j.biocon.2009.08.013

Buttler, E.I., Gilchrist, H.G., Descamps, S., Forbes, M.R., Soos, C., 2011. Handling stress of female common eiders during avian cholera outbreaks. J. Wildl. Manage. 75, 283–288. doi:10.1002/jwmg.38

Cade, T.J., 2007. Exposure of California condors to lead from spent ammunition. J. Wildl. Manage. 71, 2125. doi:10.2193/2007-084

Carsia, R. V., 2015. Adrenals, Sixth Edit. ed, Sturkie's Avian Physiology. Elsevier. doi:10.1016/B978-0-12-407160-5.00026-9

Chen, L., Wang, X., Zhang, X., Lam, P.K.S., Guo, Y., Lam, J.C.W., Zhou, B., 2017. Transgenerational endocrine disruption and neurotoxicity in zebrafish larvae after parental exposure to binary mixtures of decabromodiphenyl ether (BDE-209) and lead. Environ. Pollut. 230, 96–106. doi:10.1016/j.envpol.2017.06.053

Church, M.E., Gwiazda, R., Risebrough, R.W., Sorenson, K., Chamberlain, C.P., Farry, S., Heinrich, W., Rideout, B. a, Smith, D.R., 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environ. Sci. Technol. 40, 6143–50.

Collins, C., Kays, R., 2011. Causes of mortality in North American populations of large and medium-sized mammals. Anim. Conserv. 14, 474–483. doi:10.1111/j.1469-1795.2011.00458.x

Conde-Sieira, M., Valente, L.M.P., Hernández-Pérez, J., Soengas, J.L., Míguez, J.M., Gesto, M., 2018. Short-term exposure to repeated chasing stress does not induce habituation in Senegalese sole (*Solea senegalensis*). Aquaculture 487, 32–40. doi:10.1016/j.aquaculture.2018.01.003

Cornelius, J.M., Breuner, C.W., Hahn, T.P., 2012. Coping with the extremes: stress physiology varies between winter and summer in breeding opportunists. Biol. Lett. 8, 312–5. doi:10.1098/rsbl.2011.0865

Cory-Slechta, D.A., Virgolini, M.B., Rossi-George, A., Thiruchelvam, M., Lisek, R., Weston, D., 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin. Pharmacol. Toxicol. 102, 218–27. doi:10.1111/j.1742-7843.2007.00189.x

Cyr, N.E., Michael Romero, L., 2007. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. Gen. Comp. Endocrinol. 151, 82–9. doi:10.1016/j.ygcen.2006.12.003

Davies, P.T., Sturge-Apple, M.L., Cicchetti, D., Cummings, E.M., 2007. The role of child adrenocortical functioning in pathways between interparental conflict and child maladjustment. Dev. Psychol. 43, 918–30. doi:10.1037/0012-1649.43.4.918

Denbow, D.M., Scanes, C.G., Carsia, R. V, Harvey, S., 2000. Sturkie's Avian Physiology, 5th ed. Academic Press, San Diego.

Deviche, P., Gao, S., Davies, S., Sharp, P.J., Dawson, A., 2012. Rapid stress-induced inhibition of plasma testosterone in free-ranging male rufous-winged sparrows (*Peucaea carpalis*) characterization, time course, and recovery. Gen. Comp. Endocrinol. 177, 1–8. doi:10.1016/j.ygcen.2012.02.022

Dickens, M.J., Earle, K. a, Romero, L.M., 2009. Initial transference of wild birds to captivity alters stress physiology. Gen. Comp. Endocrinol. 160, 76–83. doi:10.1016/j.ygcen.2008.10.023

Ecke, F., Singh, N.J., Arnemo, J.M., Bignert, A., Helander, B.B., Berglund, Å.M.M.M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., Hörnfeldt, B., Bro, Å.C., Holm, K., Lanzone, M., Miller, T., Nordstro, Å., Ra, J., Rodushkin, I., Ågren, E., Ho, B., 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. Environ. Sci. Technol. doi:10.1021/acs.est.6b06024

Ellenberg, U., Setiawan, A.N., Cree, A., Houston, D.M., Seddon, P.J., 2007. Elevated hormonal stress response and reduced reproductive output in Yellow-eyed penguins exposed to unregulated tourism. Gen. Comp. Endocrinol. 152, 54–63. doi:10.1016/j.ygcen.2007.02.022

Ellison, P.T., 2003. Energetics and reproductive effort. Am. J. Hum. Biol. 15, 342–51. doi:10.1002/ajhb.10152

Ferreyra, H., Beldomenico, P.M., Marchese, K., Romano, M., Caselli, A., Correa, A.I., Uhart, M., 2015. Lead exposure affects health indices in free-ranging ducks in Argentina. Ecotoxicology 24, 735–745. doi:10.1007/s10646-015-1419-7

Finkelstein, M., Kuspa, Z., Snyder, N.F., Schmitt, N.J., 2015. California condor (*Gymnogyps californianus*). Birds North Am. doi:10.2173/bna.610

Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D.R., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc. Natl. Acad. Sci. U. S. A. 109, 11449–54. doi:10.1073/pnas.1203141109

Finkelstein, M.E., George, D., Scherbinski, S., Gwiazda, R., Johnson, M., Burnett, J., Brandt, J., Lawrey, S., Pessier, a P., Clark, M., Wynne, J., Grantham, J., Smith, D.R., 2010. Feather lead concentrations and (207)Pb/(206)Pb ratios reveal lead exposure history of California condors (*Gymnogyps californianus*). Environ. Sci. Technol. 44, 2639–47. doi:10.1021/es903176w

Finkelstein, M.E., Kuspa, Z.E., Welch, A., Eng, C., Clark, M., Burnett, J., Smith, D.R., 2014. Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis. Environ. Res. 134, 270–279. doi:10.1016/j.envres.2014.07.022

Fisher, I.J., Pain, D.J., Thomas, V.G., 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biol. Conserv. 131, 421–432. doi:10.1016/j.biocon.2006.02.018

Fortin, M.C., Cory-Slechta, D.A., Ohman-Strickland, P., Yanger, T.S., Todd, A.C., Moynihan, J., Walton, J., Brooks, A., Fiedler, N., Nwankwo, C., Cory, D.A., Johnson, W., 2012. Increased lead biomarker levels are associated with changes in hormonal response to stress in occupationally exposed male participants. Environ. Health Perspect. 120, 278–283.

Fullmers, C.S., Edelsteinp, S., 1985. Lead-binding properties of intestinal calcium-binding proteins. J. Biol. Chem. 260, 6816–6819.

Gelman, A., 2008. Scaling regression inputs by dividing two standard deviations. Stat. Med. 27, 2865–2873. doi:10.1002/sim

Grémillet, D., Prudor, A., le Maho, Y., Weimerskirch, H., 2012. Vultures of the seas: hyperacidic stomachs in wandering albatrosses as an adaptation to dispersed food resources, including fishery wastes. PLoS One 7, e37834. doi:10.1371/journal.pone.0037834

Gump, B.B., Stewart, P., Reihman, J., Lonky, E., Darvill, T., Patrick, J., Granger, D.A., Parsons, P.J., 2008. Children's low-level prenatal and postnatal blood lead exposure and adrenocortical responses to acute stress in children. Environ. Health Perspect. 116, 249–255. doi:10.1289/ehp.l0391

Guyton, A.C., Hall, J.E., 2006. Textbook of Medical Physiology, 11th ed. ElsevierInc., Philadelphia.

Harvey, S., Phillips, J.G., Rees, A., Hall, T.R., 1984. Stress and adrenal function. J. Exp. Zool. 232, 633–45. doi:10.1002/jez.1402320332

Hernández-Ochoa, I., García-Vargas, G., López-Carrillo, L., Rubio-Andrade, M., Morán-Martínez, J., Cebrián, M.E., Quintanilla-Vega, B., 2005. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. Reprod. Toxicol. 20, 221–8. doi:10.1016/j.reprotox.2005.01.007

Herring, G., Eagles-Smith, C.A., Wagner, M.T., 2016. Ground squirrel shooting and potential lead exposure in breeding avian scavengers. PLoS One 11, 1–22. doi:10.1371/journal.pone.0167926

Herring, G., Ackerman, J.T., Herzog, M.P., 2012. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ. Sci. Technol. 46, 6339–6346. doi:10.1021/es300668c

Herring, G., Gawlik, D.E., 2009. Stability of Avian Fecal Corticosterone Metabolite Levels in Frozen Avian Feces. J. Wildl. Manage. 73, 1010–1013. doi:10.2193/2008-398

Hirschenhauser, K., Spreitzer, K., Lepschy, M., Kotrschal, K., Möstl, E., 2012. Excreted corticosterone metabolites differ between two galliform species, Japanese Quail and Chicken, between sexes and between urine and faecal parts of droppings. J. Ornithol. 153, 1179–1188. doi:10.1007/s10336-012-0848-9

Hoffmann, F., Kloas, W., 2016. p,p′-Dichlordiphenyldichloroethylene (p,p′-DDE) can elicit antiandrogenic and estrogenic modes of action in the amphibian *Xenopus laevis*. Physiol. Behav. 167, 172–178. doi:10.1016/j.physbeh.2016.09.012

Houston, D., Cooper, J., 1975. The digestive tract of the whiteback griffon vulture and its role in disease transmission among wild ungulates. J. Wildl. Dis. 11, 306–313.

Hunt, W., 2012. Implications of sublethal lead exposure in avian scavengers. J. Raptor Res. 46, 389–393.

Iwasa, T., Matsuzaki, T., Yano, K., Irahara, M., 2017. Gonadotropin-inhibitory hormone plays roles in stress-induced reproductive dysfunction. Front. Endocrinol. (Lausanne). 8. doi:10.3389/fendo.2017.00062

Jachowski, D.S., Washburn, B.E., Millspaugh, J.J., 2015. Revisiting the importance of accounting for seasonal and diel rhythms in fecal stress hormone studies. Wildl. Soc. Bull. 39, 738–745. doi:10.1002/wsb.592

Jankowski, M.D., Franson, J.C., Möstl, E., Porter, W.P., Hofmeister, E.K., 2010. Testing independent and interactive effects of corticosterone and synergized resmethrin on the immune response to West Nile virus in chickens. Toxicology 269, 81–8. doi:10.1016/j.tox.2010.01.010

Jankowski, M.D., Wittwer, D.J., Heisey, D.M., Franson, J.C., Hofmeister, E.K., 2009. The adrenocortical response of greater sage grouse (*Centrocercus urophasianus*) to capture, ACTH Injection, and confinement, as measured in fecal samples. Physiol. Biochem. Zool. 82, 190–201. doi:10.1086/596513.The

Johnson, C.K., Kelly, T.R., Rideout, B.A., 2013. Lead in ammunition: A persistent threat to health and conservation. Ecohealth 10, 455–464. doi:10.1007/s10393-013-0896-5

Jones, B.C., Smith, A.D., Bebus, S.E., Schoech, S.J., 2016. Two seconds is all it takes: European starlings (*Sturnus vulgaris*) increase levels of circulating glucocorticoids after witnessing a brief raptor attack. Horm. Behav. 78, 72–78. doi:10.1016/j.yhbeh.2015.10.017

Jones, R.B., Satterlee, D.G., Waddington, D., Cadd, G.G., 2000. Effects of repeated restraint in Japanese quail genetically selected for contrasting adrenocortical responses. Physiol. Behav. 69, 317–324. doi:10.1016/S0031-9384(00)00204-3

Jung-Testas, I., Baulieu, E.E., 1998. Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system. J. Steroid Biochem. Mol. Biol. 65, 243–51.

Kaushal, D., Garg, M.L., Bansal, M.R., Bansal, M.P., 1996. Biokinetics of lead in various mouse organs using radiotracer technique. Biol. Trace Elem. Res. 53, 249–260. doi:10.1007/BF02784561

Keay, J.M., Singh, J., Gaunt, M.C., Kaur, T., 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J. Zoo Wildl. Med. 37, 234–44. doi:10.1638/05-050.1

Khan, M.Z., Altmann, J., Isani, S.S., Yu, J., 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen. Comp. Endocrinol. 128, 57–64.

Koford, C.B., 1953. California Condor, 2nd ed. National Audobon Society, Dover Publications, New York.

Koren, L., Nakagawa, S., Burke, T., Soma, K.K., Wynne-Edwards, K.E., Geffen, E., 2012a. Non-breeding feather concentrations of testosterone , corticosterone and cortisol are associated with subsequent survival in wild house sparrows. Proc. R. Soc. Biol. Sci. 279, 1560–1566. doi:10.1098/rspb.2011.2062

Koren, L., Whiteside, D., Fahlman, A., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards, K., 2012b. Cortisol and corticosterone independence in cortisol-dominant wildlife. Gen. Comp. Endocrinol. 177, 113–9. doi:10.1016/j.ygcen.2012.02.020

Kouwenberg, A., McKay, D.W., Fitzsimmons, M.G., Storey, A.E., 2015. Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: feather corticosterone levels of food-supplemented Atlantic Puffin chicks. J. F. Ornithol. 86, 73–83. doi:10.1111/jofo.12090

Kurle, C.M., Bakker, V.J., Copeland, H., Burnett, J., Jones Scherbinski, J., Brandt, J., Finkelstein, M.E., 2016. Terrestrial scavenging of marine mammals: cross-ecosystem contaminant transfer and potential risks to endangered California condors (*Gymnogyps californianus*). Environ. Sci. Technol. acs.est.6b01990. doi:10.1021/acs.est.6b01990

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149. doi:10.1016/j.ygcen.2006.02.013

Lanphear, B.P., Rauch, S., Auinger, P., Allen, R.W., Hornung, R.W., 2018. Low-level lead exposure and mortality in US adults: a population-based cohort study. Lancet Public Heal. 2667, 1–8. doi:10.1016/S2468-2667(18)30025-2

Lèche, A., Busso, J.M., Navarro, J.L., Hansen, C., Marin, R.H., Martella, M.B., 2011. Non-invasive monitoring of adrenocortical activity in Greater Rhea (Rhea americana) by measuring fecal glucocorticoid metabolites. J. Ornithol. 152, 839–847. doi:10.1007/s10336-011-0661-x

Legagneux, P., Gauthier, G., Chastel, O., Picard, G., Bêty, J., 2011. Do glucocorticoids in droppings reflect baseline level in birds captured in the wild? A case study in snow geese. Gen. Comp. Endocrinol. 172, 440–445. doi:10.1016/j.ygcen.2011.04.009

López-Jiménez, L., Blas, J., Tanferna, A., Cabezas, S., Marchant, T., Hiraldo, F., Sergio, F., 2017. Lifetime variation in feather corticosterone levels in a long-lived raptor. Oecologia 183. doi:10.1007/s00442-016-3708-0

Love, O.P., Breuner, C.W., Vézina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. Horm. Behav. 46, 59–65. doi:10.1016/j.yhbeh.2004.02.001

Lucchini, R.G., Zoni, S., Guazzetti, S., Bontempi, E., Micheletti, S., Broberg, K., Parrinello, G., Smith, D.R., 2012. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. Environ. Res. 118, 65–71. doi:10.1016/j.envres.2012.08.003

Lukacs, P.M., Burnham, K.P., Anderson, D.R., 2010. Model selection bias and Freedman ' s paradox 117–125. doi:10.1007/s10463-009-0234-4

Madliger, C.L., Semeniuk, C.A.D., Harris, C.M., Love, O.P., 2015. Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker. Biol. Conserv. 192, 409–417. doi:10.1016/j.biocon.2015.10.021

Martin, L.B., 2009. Stress and immunity in wild vertebrates: timing is everything. Gen. Comp. Endocrinol. 163, 70–6. doi:10.1016/j.ygcen.2009.03.008

Martinez-Haro, M., Taggart, M. a, Green, A.J., Mateo, R., 2009. Avian digestive tract simulation to study the effect of grit geochemistry and food on Pb shot bioaccessibility. Environ. Sci. Technol. 43, 9480–6. doi:10.1021/es901960e

Mazerolle, M.J., 2017. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)]. R Packag. version 2.1-1. https://cran.rproject.org/package=AICcmodavg

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15. doi:10.1016/S0018-506X(02)00024-7

Meaney, M., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J.R., Plotsky, P.M., 1996. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev. Neurosci. 18, 49–72.

Meillère, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., Angelier, F., 2016. Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*). Sci. Total Environ. 566–567, 93–101. doi:10.1016/j.scitotenv.2016.05.014

Miller, R., Plessow, F., Rauh, M., Gröschl, M., Kirschbaum, C., 2013. Comparison of salivary cortisol as measured by different immunoassays and tandem mass spectrometry. Psychoneuroendocrinology 38, 50–57. doi:10.1016/j.psyneuen.2012.04.019

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol. 138, 189–99. doi:10.1016/j.ygcen.2004.07.002

Möstl, E., Rettenbacher, S., Palme, R., 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Ann. N. Y. Acad. Sci. 1046, 17–34. doi:10.1196/annals.1343.004

Nakagawa, S., Möstl, E., Waas, J., 2003. Validation of an enzyme immunoassay to measure faecal glucocorticoid metabolites from Adelie penguins (*Pygoscelis adeliae*): a non-invasive tool for estimating. Polar Biol. 26, 491–493. doi:10.1007/s00300-003-0506-z

Newman, A.E.M., Hess, H., Woodworth, B.K., Norris, D.R., 2017. Time as tyrant: the minute, hour and day make a difference for corticosterone concentrations in wild nestlings. Gen. Comp. Endocrinol. 250, 80–84. doi:10.1016/j.ygcen.2017.05.022

Nilsson, P.B., Hollmén, T.E., Atkinson, S., Mashburn, K.L., Tuomi, P.A., Esler, D., Mulcahy, D.M., Rizzolo, D.J., 2008. Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (*Histrionicus histrionicus*). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 149, 275–283. doi:10.1016/j.cbpa.2008.01.002

Oomen, a G., Tolls, J., Sips, a J. a M., Van den Hoop, M. a G.T., 2003. Lead speciation in artificial human digestive fluid. Arch. Environ. Contam. Toxicol. 44, 107–15. doi:10.1007/s00244-002-1225-0

Orchinik, M., Moore, F.L., Rose, J.D., 1994. Mechanistic and functional studies of rapid corticosteroid actions. Ann. N. Y. Acad. Sci. 746, 101-112-114. doi:10.1111/j.1749-6632.1994.tb39219.x

Ouyang, J.Q., Quetting, M., Hau, M., 2012. Corticosterone and brood abandonment in a passerine bird. Anim. Behav. 84, 261–268. doi:10.1016/j.anbehav.2012.05.006

Owen, J.C., Nakamura, A., Coon, C.A., Martin, L.B., 2012. The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). Vet. Res. 43, 34. doi:10.1186/1297-9716-43-34

Pain, D., 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. Ingestion Lead from Spent Ammunit. Implic. Wildl. Humans 99–118. doi:10.4080/ilsa.2009.0108

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann. N. Y. Acad. Sci. 1040, 162–71. doi:10.1196/annals.1327.021

Pokras, M. a, Kneeland, M.R., 2008. Lead poisoning: using transdisciplinary approaches to solve an ancient problem. Ecohealth 5, 379–85. doi:10.1007/s10393-008-0177-x

Provencher, J.F., Forbes, M.R., Hennin, H.L., Love, O.P., Braune, B.M., Mallory, M.L., Gilchrist, H.G., 2016. Implications of mercury and lead concentrations on breeding physiology and phenology in an Arctic bird. Environ. Pollut. 218, 1014–1022. doi:10.1016/j.envpol.2016.08.052

R Core Development Team, 2011. R: A Language and Environment for Statistical Computing.

Raff, H., Homar, P.J., Burns, E.A., 2002. Comparison of two methods for measuring salivary cortisol. Clin Chem 48, 207–208.

Rattner, B. a, 2009. History of wildlife toxicology. Ecotoxicology 18, 773–83. doi:10.1007/s10646-009-0354-x

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., Palme, R., 2004. Measurement of corticosterone metabolites in chicken droppings. Br. Poult. Sci. 45, 704–11. doi:10.1080/00071660400006156

Rideout, B.A., Stalis, I., Papendick, R., Pessier, A., Puschner, B., Finkelstein, M.E., Smith, D.R., Johnson, M., Mace, M., Stroud, R., Brandt, J., Burnett, J., Parish, C., Petterson, J., Witte, C., Stringfield, C., Orr, K., Zuba, J., Wallace, M., Grantham, J., 2012. Patterns of mortality in free-ranging California Condors (Gymnogyps californianus). J. Wildl. Dis. 48, 95–112.

Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.

Romero, L.M., Fairhurst, G.D., 2016. Measuring corticosterone in feathers: Strengths, limitations, and suggestions for the future. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 202, 112–122. doi:10.1016/j.cbpa.2016.05.002

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 73–79. doi:10.1016/j.cbpb.2004.11.004

Rossi-George, A., Virgolini, M.B., Weston, D., Cory-Slechta, D.A., 2009. Alterations in glucocorticoid negative feedback following maternal Pb, prenatal stress and the combination: a potential biological unifying mechanism for their corresponding disease profiles. Toxicol. Appl. Pharmacol. 234, 117–27. doi:10.1016/j.taap.2008.10.003

Sabolić, I., 2006. Common mechanisms in nephropathy induced by toxic metals. Nephron. Physiol. 104, p107-14. doi:10.1159/000095539

Sanders, T., Liu, Y., Buchner, V., Tchounwou, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Rev. Environ. Health 24, 15–45.

Schmidt, K.L., Soma, K.K., 2008. Cortisol and corticosterone in the songbird immune and nervous systems: local vs. systemic levels during development. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R103-10. doi:10.1152/ajpregu.00002.2008

Schoech, S.J., Ketterson, E.D., Nolan, V., Jr, V.N., 1999. Exogenous testosterone and the adrenocortical response in dark-eyed juncos. Auk 116, 64–72. doi:10.2307/4089454

Schoech, S.J., Romero, L.M., Moore, I.T., Bonier, F., 2013. Constraints, concerns and considerations about the necessity of estimating free glucocorticoid concentrations for field endocrine studies. Funct. Ecol. 27, 1100–1106. doi:10.1111/1365-2435.12142

Schoenle, L.A., Dudek, A.M., Moore, I.T., Bonier, F., 2017. Hormones and Behavior Red-winged blackbirds (*Agelaius phoeniceus*) with higher baseline glucocorticoids also invest less in incubation and clutch mass. Horm. Behav. 90, 1–7. doi:10.1016/j.yhbeh.2017.02.002

Shanks, N., Meaney, J., 1995. Neonatal Endotoxin Exposure Responsivity to Stress Alters the Development of the Axis : Early Illness and Later 15.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166, 869–87. doi:10.1007/s00442-011-1943-y

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? Gen. Comp. Endocrinol. 166, 614–9. doi:10.1016/j.ygcen.2009.12.017

Snyder, N., Snyder, H., 2000. The California Condor: A Saga of Natural History & Conservation, Bird-Banding. Academic Press, San Diego. doi:10.2307/4510445

Stahn, C., Buttgereit, F., 2008. Genomic and nongenomic effects of glucocorticoids. Nature, Clin. Pract. Rheimatology 4, 525–33. doi:10.1038/ncprheum0898

Staley, A.M., Blanco, J.M., Dufty, A.M., Wildt, D.E., Monfort, S.L., 2007. Fecal steroid monitoring for assessing gonadal and adrenal activity in the golden eagle and peregrine falcon. J. Comp. Physiol. B. 177, 609–22. doi:10.1007/s00360-007-0159-2

Tartu, S., Lendvai, Á.Z., Blévin, P., Herzke, D., Bustamante, P., Moe, B., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015. Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*). Gen. Comp. Endocrinol. 219, 165–172. doi:10.1016/j.ygcen.2014.12.018

Tartu, S., Angelier, F., Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Gabrielsen, G.W., Verboven, N., Verreault, J., Labadie, P., Budzinski, H., Wingfield, J.C., Chastel, O., 2015a. Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species. Environ. Pollut. 197, 173–180. doi:10.1016/j.envpol.2014.12.007

Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015b. Corticosterone, prolactin and egg

neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. doi:10.1016/j.scitotenv.2014.10.008

Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated: Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476–477, 553–560. doi:10.1016/j.scitotenv.2014.01.060

Telisman, S., Colak, B., Pizent, A., Jurasović, J., Cvitković, P., 2007. Reproductive toxicity of low-level lead exposure in men. Environ. Res. 105, 256–66. doi:10.1016/j.envres.2007.05.011

Tempel, D., Gutiérrez, R., 2004. Factors related to fecal corticosterone levels in California spotted owls: implications for assessing chronic stress. Conserv. Biol. 18, 538–547. doi:10.1111/j.1523-1739.2004.00372.x

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046, 54–74. doi:10.1196/annals.1343.006

United States Fish and Wildlife Service, 2013. California Condor 5-Year Review : Summary and Evaluation.

Virgolini, M.B., Bauter, M.R., Weston, D.D., Cory-Slechta, D.A., 2006. Permanent alterations in stress responsivity in female offspring subjected to combined maternal lead exposure and/or stress. Neurotoxicology 27, 11–21. doi:10.1016/j.neuro.2005.05.012

Virgolini, M.B., Chen, K., Weston, D.D., Bauter, M.R., Cory-Slechta, D.A., 2005. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol. Sci. 87, 469–82. doi:10.1093/toxsci/kfi269

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: Health implications for indigenous residents. Environ. Pollut. 234, 279–287. doi:10.1016/j.envpol.2017.11.054

Walker, B.G., Boersma, P.D., Wingfield, J.C., 2005. Field endocrinology and conservation biology. Integr. Comp. Biol. 45, 12–8. doi:10.1093/icb/45.1.12

Walker, B.G., Dee Boersma, P., Wingfield, J.C., 2006. Habituation of adult Magellanic Penguins to human visitation as expressed through behavior and corticosterone secretion. Conserv. Biol. 20, 146–154. doi:10.1111/j.1523-1739.2005.00271.x

Warnken, T., Huber, K., Feige, K., 2016. Comparison of three different methods for the quantification of equine insulin. BMC Vet. Res. 12, 196. doi:10.1186/s12917-016-0828-z

Washburn, B.E., Morris, D.L., Millspaugh, J.J., Faaborg, J., Schulz, J.H., 2002. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. Condor 104, 558–563.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A Generalized Fecal Glucocorticoid Assay for Use in a Diverse Array of Nondomestic Mammalian and Avian Species. Gen. Comp. Endocrinol. 120, 260–275. doi:10.1006/gcen.2000.7557

Whirledge, S., Cidlowski, J.A., 2010. Glucocorticoids, stress, and fertility. Minerva Endocrinol. 35, 109–125. doi:10.1586/eem.10.1

White, L.D., Cory-Slechta, D. a, Gilbert, M.E., Tiffany-Castiglioni, E., Zawia, N.H., Virgolini, M., Rossi-George, A., Lasley, S.M., Qian, Y.C., Basha, M.R., 2007. New and evolving concepts in the neurotoxicology of lead. Toxicol. Appl. Pharmacol. 225, 1–27. doi:10.1016/j.taap.2007.08.001

Wilcoxen, T.E., Boughton, R.K., Bridge, E.S., Rensel, M. a, Schoech, S.J., 2011. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. Gen. Comp. Endocrinol. 173, 461–6. doi:10.1016/j.ygcen.2011.07.007

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42, 600–9. doi:10.1093/icb/42.3.600

Wingfield, J.C., Ramenofsky, M., Pottinger, T.G., 1999. Stress Physiology in Animals, 1st ed. CRC Press LLC, Boca Raton.

CHAPTER 2: LINKING CASES OF ILLEGAL SHOOTINGS OF THE ENDANGERED CALIFORNIA CONDOR USING STABLE LEAD ISOTOPES

Myra E. Finkelstein, Zeka E. Kuspa, Alacia Welch, Curtis Eng, Michael Clark, Joseph Burnett, Donald R. Smith

Abstract

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

knowledge, this is the first study to use lead isotopes as a tool to retrospectively link wildlife shooting events.

1. Introduction

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

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

California condors are routinely lead poisoned from feeding on carcasses contaminated by spent lead ammunition and require ongoing intensive management and supportive care to prevent lead-related mortalities (Church et al., 2006; Finkelstein et al., 2012; Parish et al., 2009; Walters et al., 2010). In addition to deaths from lead poisoning, condors face other threats such as morbidity/mortality from gunshot; since 1992 four condors have died as a result of gunshot wounds (Rideout et al., 2012). The shooting of nongame wildlife is illegal and punishable by fines of up to $2,000 (Californnia Rules of Court, 2011). The shooting of a federally recognized endangered species triggers an additional violation of federal law punishable by a fine of up to $50,000 or 1 year imprisonment (US Fish and Wildlife Service, 2003). Enforcement of illegal shooting laws may also receive high priority in cases involving endangered species, as each incidence of injury or death could jeopardize the success of publicly-funded endangered species recovery programs.

All free-ranging condors in California are fit with radio and/or GPS transmitters to monitor their movements on a near daily basis. Condors are recaptured approximately twice per year for health and lead exposure monitoring as well as tag/transponder maintenance, and more frequently if injury or risk of lead poisoning is suspected. Field screening of blood lead levels (LeadCare I and II field measurement kits) followed by measurement through an accredited laboratory and archiving of blood samples for possible stable lead isotope analyses are standard procedure. Between March and October 2009 three California condors independently presented with lead poisoning and were transported to the Gottlieb Animal Health and Conservation Center (LA Zoo, California, USA) for clinical management, including chelation therapy. All three birds were identified via

radiograph to possess birdshot embedded in their tissues, indicating they had been shot. After the second of the three cases was discovered, efforts were undertaken to identify the person(s) responsible for the shootings, including the offering of a $40,500 reward for information leading to the arrest and conviction of the perpetrator(s) (Sahagun, 2009). However, as of May 2014, little to no information about the circumstances surrounding the shooting(s) has surfaced and no arrests have been made.

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

2. Materials and Methods

2.1. Study subjects and sample collections (See also Table A1 for detailed timeline of events and Appendix A for sample collection details).

Illegal shooting event case study. This study presents cases of three California condors (Studbook IDs 286, 375, and 401) who were independently recaptured at trapping sites in central California, found to be lead poisoned with

blood lead values of “High” (LeadCare field collection kit), and transported to the LA Zoo for radiographs [Eklin EDR6 Digital Radiograph System (Rapid Start)] and clinical management (e.g., chelation therapy) per standard procedure. All three birds were discovered to contain multiple embedded birdshot pellets (condor 286 on 4 March 2009 with 10 birdshot pellets, condor 375 on 26 March 2009 with three birdshot pellets, and condor 401 on 30 October 2009 with four birdshot pellets). Based on field observations preceding presentation at the LA Zoo, all three condors were capable of flight and displayed no outward signs of traumatic injury; examinations within the clinic indicated that all pellet entry wounds had healed by the time of radiographic discovery. Radiographic and clinical exams showed that birdshot in condor 375 and 401 was embedded in their soft tissues (muscle, coelomic cavity, gastrointestinal tract, etc.) and not in the joint and/or bone; for 286, the radiographic and clinical exams indicated the birdshot was most likely embedded in soft tissue, yet this assessment was not definitive. Birdshot were recovered surgically (375 = one pellet, condor 401 = one pellet) or post-mortem (286 = five pellets). At the same time, condor tissues (blood, feathers) were collected from all three condors, or in the case of 401’s feather, marked for future collection once grown-in. Condor 286 died of lead toxicosis on 11 May 2009 (Rideout et al., 2012) and samples of liver, kidney, and bone were collected at necropsy. Condor 401 had additional blood and feather tissue samples collected on 12 April and 27 May 2009.

Condor 401 - Ingested buckshot. On 21 June 2010 condor 401 again presented with lead poisoning (blood lead value of “High”, LeadCare field collection kit) and was transported to the LA Zoo for treatment where radiographs revealed a buckshot pellet in the bird’s gastrointestinal tract; the buckshot was subsequently

collected following regurgitation and a second previously identified embedded birdshot pellet was surgically removed from the bird's soft tissue. Tissue samples were collected (blood) or marked for future collection (feather) at the time the bird presented with lead poisoning.

2.2. Sample Processing and Analysis

Biological and birdshot/buckshot pellet samples were processed and analyzed using established trace metal clean techniques, as described elsewhere (Finkelstein et al., 2012; Finkelstein et al., 2010; Finkelstein et al., 2003; Gwiazda et al., 2005; Smith et al., 1996). For primary feathers, individual sections of feather vane (~2 cm width along rachis axis) were treated as separate samples; each feather section was weighed and then processed under trace metal clean conditions to remove surface contamination by washing sequentially with acetone, ultrapure water, 1% HNO_3 and ultrapure water, as previously reported (Church et al., 2006; Finkelstein et al., 2010). All biological samples (feather, whole blood, liver, kidney, and bone) were processed as described previously (Finkelstein et al., 2010; Finkelstein et al., 2003; Gwiazda et al., 1998; Smith et al., 1996); briefly, samples were digested overnight in 2 mL sub-boiling concentrated HNO_3 in closed Teflon vials, evaporated to dryness, and reconstituted in 1% HNO_3 for analysis. Birdshot/buckshot pellets were individually cleaned and then leached in 1 mL 1% HNO_3 for 30 sec for analyses, as previously described (Finkelstein et al., 2012).

Sample lead concentrations and isotope ratios were determined by inductively coupled plasma mass spectrometry (ICP-MS, Finnigan MAT Element magnetic sector), measuring masses of ^{206}Pb, ^{207}Pb, and ^{208}Pb as previously

described (Finkelstein et al., 2003; Gwiazda et al., 1998). Added ^{205}Tl was used as an internal standard. The precision of the lead isotope ratio measurements was ~0.10% (2 x the relative standard deviation, 2 RSD), based on condor tissue samples analyzed in triplicate within an analytical run. Between-run (i.e., long-term over several years) measurement precision was <0.20% (2 RSD), based on repeated measurements of blood and lead ammunition leachate samples. Isotope ratios (^{207}Pb /^{206}Pb) that differed by <0.20% (i.e., the 2 RSD of long-term measurement precision) were considered measurably indistinguishable.

3. Results and Discussion

3.1 Overview

The discovery of the embedded birdshot in condor 375, 3 weeks after condor 286 similarly presented with embedded birdshot, initiated a high priority analytical assessment of the biological samples associated with these cases (Fig. 1). Within ~2 months we determined that the birdshot removed from condors 286 and 375 had lead isotope ratios that were measurably indistinguishable from one another. Lead concentrations and isotopic compositions were then measured in stored blood and feather tissues from these two birds, as well as in samples from condor 401 after the discovery ~8 months later (Oct 2009) of embedded birdshot indicating that this condor had also been shot (Fig. 1, see Table A1 for timeline details). While California condors are monitored on a near daily basis, with many birds being tracked by satellite telemetry (Walters et al., 2010), none of the three birds (condors 286, 375, or 401) were fitted with a satellite transmitter during the timeframe of the presumed shooting(s). Furthermore, the near daily tracking data collected by field biologists did

not provide sufficient information about the locations or spatial associations of these birds that could be used to infer the timing or location of the shooting(s).

Based on the preponderance of lead concentration and isotopic composition evidence from blood, feather, and birdshot pellet samples, we propose that all three condors were shot in a common shooting event. While none of the three birds died from their gunshot wounds, two of the birds (condors 286 and 375) were poisoned by a lead source with a ^{207}Pb /^{206}Pb ratio that was measurably indistinguishable from the embedded birdshot. Notably, one of these birds (condor 286) died from lead toxicosis (Rideout et al., 2012) as a result of this lead poisoning event on 11 May 2009.

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

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

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

A total of eight embedded birdshot pellets were recovered from condors 286 (n = 5), 375 (n = 1), and 401 (n = 2) during necropsy (condor 286) or treatment for lead poisoning (condors 375 and 401) between April 2009 and June 2010 (Table A1). All eight pellets were similar in appearance and their lead isotopic compositions

were measurably indistinguishable from one another (average $^{207}Pb/^{206}Pb = 0.8188 \pm 0.0005$ SD, n=8, Table A2). The range of diameters and weights for the pellets (2.61-2.97 mm and 105-136 mg, respectively) are consistent with either #6 or #7 birdshot. However, since the mass and shape of these pellets have most certainly been altered from being fired and striking the birds, with some of the pellets appearing to have tissue residue on their surface that may affect these measurements, the range in diameter and mass reported above is not unexpected but precludes a more precise classification of the shot.

Condor 286 and 375 presented with lead poisoning (blood lead levels of 155 and 180 µg/dL on 4 March and 26 March 2009, respectively) when they were identified via radiograph to contain embedded birdshot in their tissues. For 286, the $^{207}Pb/^{206}Pb$ ratio of the blood sample collected at the time he presented with lead poisoning was measurably indistinguishable from the $^{207}Pb/^{206}Pb$ ratios of the five birdshot pellets recovered from his tissues (blood $^{207}Pb/^{206}Pb = 0.8194$, birdshot $^{207}Pb/^{206}Pb = 0.8183 - 0.8194$). Condor 375's blood isotope ratios were very similar to, yet measurably different from, the single birdshot pellet recovered from her tissues (blood $^{207}Pb/^{206}Pb = 0.8225$ and 0.8248, recovered birdshot $^{207}Pb/^{206}Pb = 0.8184$, Table A2). In both cases, the data suggest either that the source of lead poisoning was the embedded birdshot and/or ingestion of a lead source (unrecovered) that was isotopically similar to the embedded birdshot.

Condor 401 on 12 April 2009 was found to have a field blood lead level that was elevated (11.1 µg/dL, LeadCare field collection kit) (Cade, 2007) but below the threshold indicating clinical treatment (35 µg/dL); thus, condor 401's blood sample was archived per established protocol (Appendix A). Condor 401's archived blood

sample was prioritized for analysis after he presented ~8 mos. later (30 October 2009) with lead poisoning (86 µg/dL, Louisiana Animal Disease Diagnostic Laboratory) and identified via radiograph to contain tissue-embedded birdshot. The $^{207}Pb/^{206}Pb$ ratio of condor 401's 12 April 2009 blood sample ($^{207}Pb/^{206}Pb$ blood = 0.8166, lead concentration 16.6 µg/dL) was measurably indistinguishable from his recovered birdshot (average $^{207}Pb/^{206}Pb$ = 0.8187, n = 2, Table A2).

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

We have previously established that feathers can be used to reconstruct a condor's lead exposure history over the 3 - 4 month timeframe of feather growth, and that the relationship between blood lead (µg/dL) and feather lead (µg/g) concentrations (i.e., blood lead:feather lead ratio) is ~19:1 (Finkelstein et al., 2010). Here we used this approach to reconstruct the lead exposure histories of condors 286, 375 and 401. Fortuitously, these three condors had primary feathers that were growing over the period that blood samples were collected and near the time of the suspected shooting(s) (Fig. 1, Fig. 2).

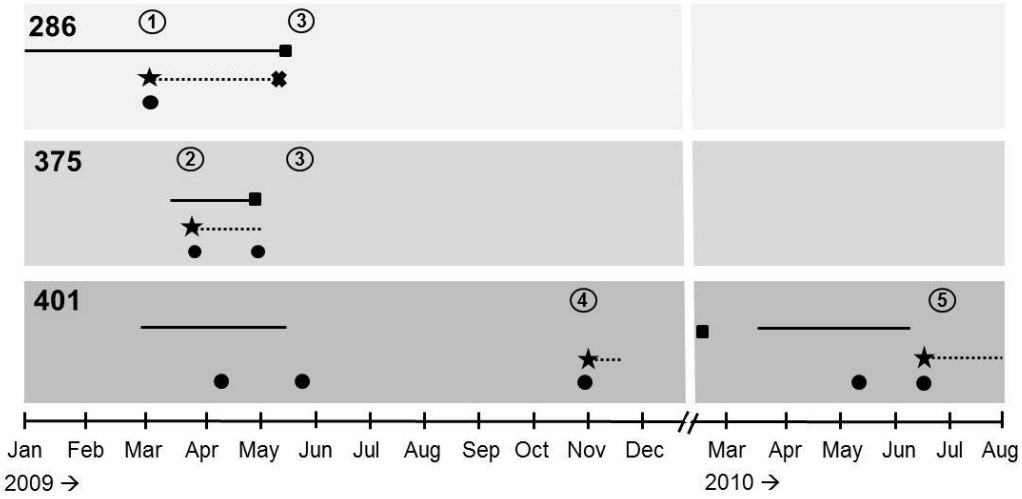

Figure. 1. Timeline of condor feather growth (estimated), sample collection, and analysis for the illegal shooting cases of California condors 286, 375, and 401 (see also Table A1). Event ①, 5 March 2009, radiograph of condor 286 revealed birdshot embedded in his tissues. Event ②, 26 March 2009, radiograph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

3.2.2.1. Feather lead concentrations.

The feather lead concentration profile from condor 286 indicates that the bird was initially lead exposed at the end of January 2009 with lead concentrations reaching a peak of 39 µg/g by mid-February (equivalent to ~730 µg/dL estimated blood lead). For condor 375, the feather did not start growing until after this bird's peak lead exposure had occurred, as evidenced by a feather lead concentration profile that is clearly decreasing from a prior acute exposure event; that prior exposure event appears to be of a similar magnitude to condor 286's peak exposure (Fig. 2A). Similarly, condor 401's feather also did not start growing until after the estimated time of 286's lead poisoning (end of January 2009) with the lead concentration profile indicating that condor 401 did not experience an acute lead poisoning event as evidenced in condors 286 and 375 (Fig. 2A). Rather, condor 401's feather lead concentration profile illustrates he was moderately lead exposed at the time the feather started growing with a feather lead concentration of 1.45 µg/g (equivalent to 27 µg/dL estimated blood lead).

Given that the feathers from condors 286 and 375 illustrate these birds were exposed to an acute lead poisoning event (Fig. 2A), and that the $^{207}Pb/^{206}Pb$ ratios of their blood samples at the time they presented with lead poisoning were very similar to their embedded shot (Fig. 2B), we propose that the most plausible cause of lead poisoning in these two birds was from ingestion of a lead source with an isotopic signature of their embedded pellets. This may have occurred either through feeding on a carcass that was contaminated with birdshot identical to the bird's tissue-embedded pellets, or from ingestion of tissue-embedded pellet(s) that they preened

from their wounds. In contrast, we propose that condor 401, with a moderately elevated blood lead level, was exposed only from the tissue-embedded birdshot - a suggestion supported by a study in humans that reported elevated blood lead levels (range 7-50 µg/dL) in patients with embedded lead shrapnel in their tissues (Farrell et al., 1999).

3.2.2.2. Feather lead isotopic compositions.

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

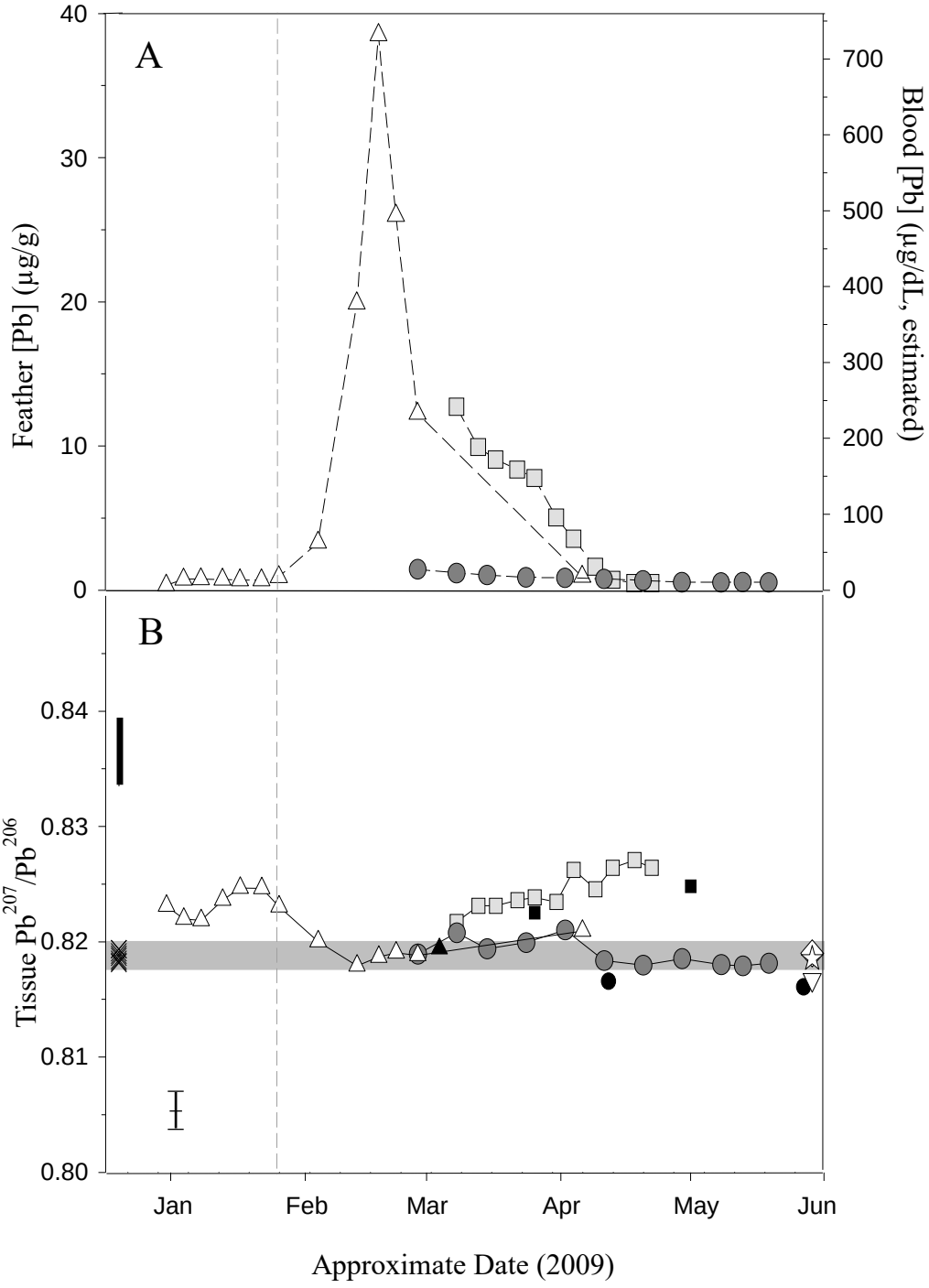
A
B
Feather [Pb] (µg/g)
Blood [Pb] (µg/dL, estimated)
Tissue Pb207/Pb206
Approximate Date (2009)
Jan
Feb
Mar
Apr
May
Jun
0
10
20
30
40
0
100
200
300
400
500
600
700
0.80
0.81
0.82
0.83
0.84

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

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

Condor 401 did not experience an acute lead poisoning event during the timeframe of feather growth (Fig. 2A); the feather lead concentration profile indicates the bird had experienced a moderate lead exposure event (estimated blood lead of 27 µg/dL, Fig. 2A) that we attribute to lead from the tissue-embedded shot, as noted previously. While condor 401 was not severely lead poisoned, the $^{207}Pb/^{206}Pb$ ratios of both the feather and blood were measurably indistinguishable from the recovered birdshot pellets, indicating that condor 401's tissue lead isotopic signature was heavily influenced by this moderate lead exposure event (Fig. 2B).

3.2.3. Commonalities between condor shooting events and study limitations.

Given the preponderance of evidence presented above (see also Table 1), we conclude that the three cases of condor shootings are linked, and possibly from a

single shooting event. This conclusion is supported by: 1) the $^{207}Pb/^{206}Pb$ ratios of recovered birdshot and blood (condors 286 and 401) and feather (condor 286, 375, and 401) samples were measurably indistinguishable from one another, and 2) the feather lead concentration and isotopic composition profiles are consistent with the suggestion that condors 286, 375 and 401 were exposed to an elevated lead source within the same timeframe.

While we consider this the most likely scenario, this conclusion may be qualified by several limitations of the study. First, unlike the feather from condor 286, the feathers collected from condors 375 and 401 did not start growing until after the estimated time of exposure, and so their feather lead concentration and isotopic composition profiles did not capture the peak exposure event. Nonetheless, the feather lead profiles of condors 375 and 401 are consistent with, and in fact cannot exclude, a lead exposure event in late January 2009 to a lead source common across all three cases. Second, the fact that the lead isotope signatures in the condor tissues and the recovered birdshot were measurably indistinguishable from one another does not by itself prove that the lead poisonings in all three birds arose from a single source of birdshot. There has not been a systematic evaluation of the isotopic variability of birdshot within a single shotshell cartridge, between cartridges within a box, or between boxes within or across manufacturers for birdshot ammunition available within the central California range. However, we have evaluated the isotopic composition of lead bullet and shotgun ammunition from California (n=73) (Finkelstein et al., 2012), and only five ammunition samples (~7%) had lead isotopic compositions that fell within the range of the recovered embedded birdshot from the three condors (Fig. 3). Further, Scheuhammer and Templeton

(1998) reported that “The within-cartridge variability in the ^{206}Pb:^{207}Pb ratios for pellets taken from the same shotshell cartridge was very low (CV <0.5%) and this was also true for pellets from different cartridges from the same box (CV < 0.3%). Although there was considerable variability in the ^{206}Pb:^{207}Pb ratios between different brands of shot…”. Thus, in total, the evidence presented suggests that the birds were shot by a common source at the same time, although we cannot exclude the possibility that three independent shooting events occurred within the same timeframe with shot that was measurably indistinguishable from each other.

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

Condor 401 presented again with clinical lead poisoning in June 2010 (blood lead 556 µg/dL), when a radiograph showed an ingested buckshot within the bird’s gastrointestinal tract as well as the three remaining embedded birdshot pellets that had been discovered the previous year (Fig. A1). The $^{207}Pb/^{206}Pb$ ratio (0.8130) of condor 401’s blood collected at the time of acute lead poisoning on 21 June 2010 was measurably indistinguishable from the lead isotope ratio of the recovered ingested buckshot ($^{207}Pb/^{206}Pb$ = 0.8122) and most recently grown feather section ($^{207}Pb/^{206}Pb$ = 0.8142) coinciding with the peak of exposure (Fig. 4). Of significance is that the isotopic composition of the buckshot was measurably different from the recovered embedded birdshot (average birdshot $^{207}Pb/^{206}Pb$ = 0.8188, Fig. 4B, Table A2).

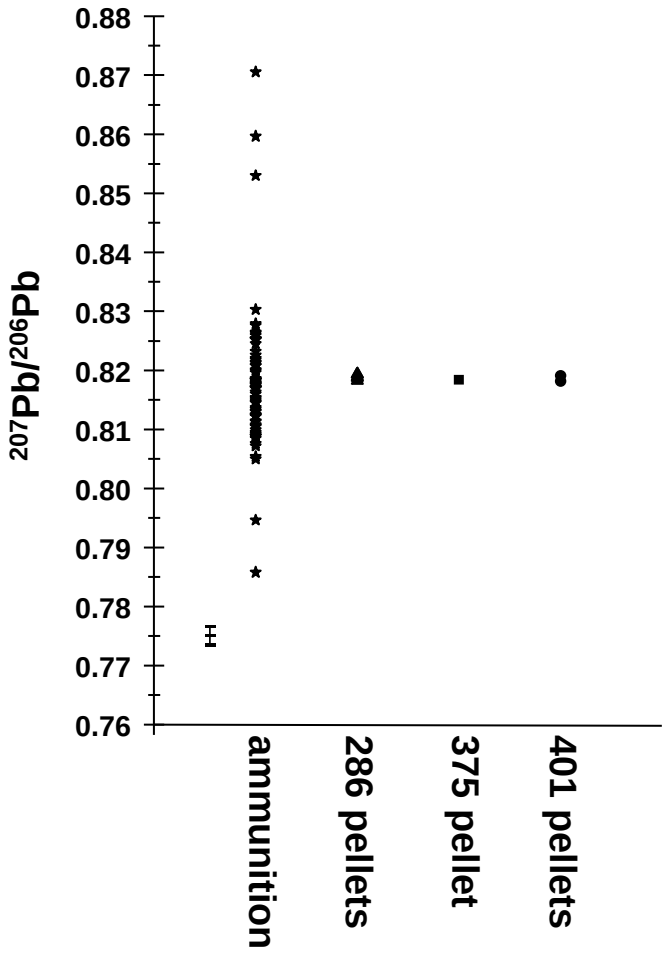

Figure 3. $^{207}Pb/^{206}Pb$ ratios of the embedded birdshot pellets recovered from condors 286 (▲ , range = 0.8183 - 0.8194, n=5), **375** (■, 0.8184, n=1), **401** (● , 0.8182, 0.8191, n=2), **and ammunition samples from California** [★, range = 0.7858 - 0.8706, n=73, from Finkelstein et al. (2012)]. Only five out of 73 ammunition samples (~7%) fall within the range of the recovered embedded birdshot pellets, supporting our suggestion that the recovered birdshot pellets originated from a common shooting event. The error bar in lower left reflects the $^{207}Pb/^{206}Pb$ measurement error (i.e., 2RSD, see methods).

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

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

Evidence	Condor			Interpretation
	286	375	401	
Feather $^{207}Pb/^{206}Pb$ ratio profiles show exposures were to a lead source measurably indistinguishable isotopically from the recovered embedded birdshot	$^{207}Pb/^{206}Pb$ ratio of peak exposure measurably indistinguishable from birdshot	$^{207}Pb/^{206}Pb$ ratio in first to grow feather segment measurably indistinguishable from birdshot	$^{207}Pb/^{206}Pb$ ratios of feather segments measurably indistinguishable from birdshot	Feather $^{207}Pb/^{206}Pb$ ratio profiles are consistent with an acute (condors 286, 375) or moderate (condor 401) exposure event that occurred in late January from either ingested (condos 286, 375) or embedded (condor 401) birdshot

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

Condor 401's feather lead concentration profile captured the May/June 2010 severe lead poisoning event that is attributed to the ingested buckshot, with a peak feather lead concentration of 53.7 µg/g, corresponding to an estimated blood lead of over 1000 µg/dL (Fig. 4A). Condor 401's feather completed growing soon after this acute exposure event and does not reflect the decline in body lead levels associated with clinical chelation treatment in June 2010. Noteworthy is that condor 401's feather also showed evidence of an additional lead exposure event, likely several weeks to months before the feather started growing in mid-March 2010 (Fig. 4). While the peak lead exposure from this event occurred prior to feather growth, the oldest (i.e., first-to-grow) feather segment had a lead concentration (3.36 µg/g) that corresponds to an estimated blood lead level >60 µg/dL, which is above the blood lead threshold indicating clinical chelation for condors (~35 µg/dL). Therefore, blood and feather analyses show that condor 401 experienced at least four lead poisoning events (blood lead >35 µg/dL) throughout the ~1.5 year study period, with the bird being hospitalized for clinical treatment of lead poisoning three times (see also Table A1), a finding underscoring the epidemic lead poisoning rates observed in condors in California (Finkelstein et al., 2012).

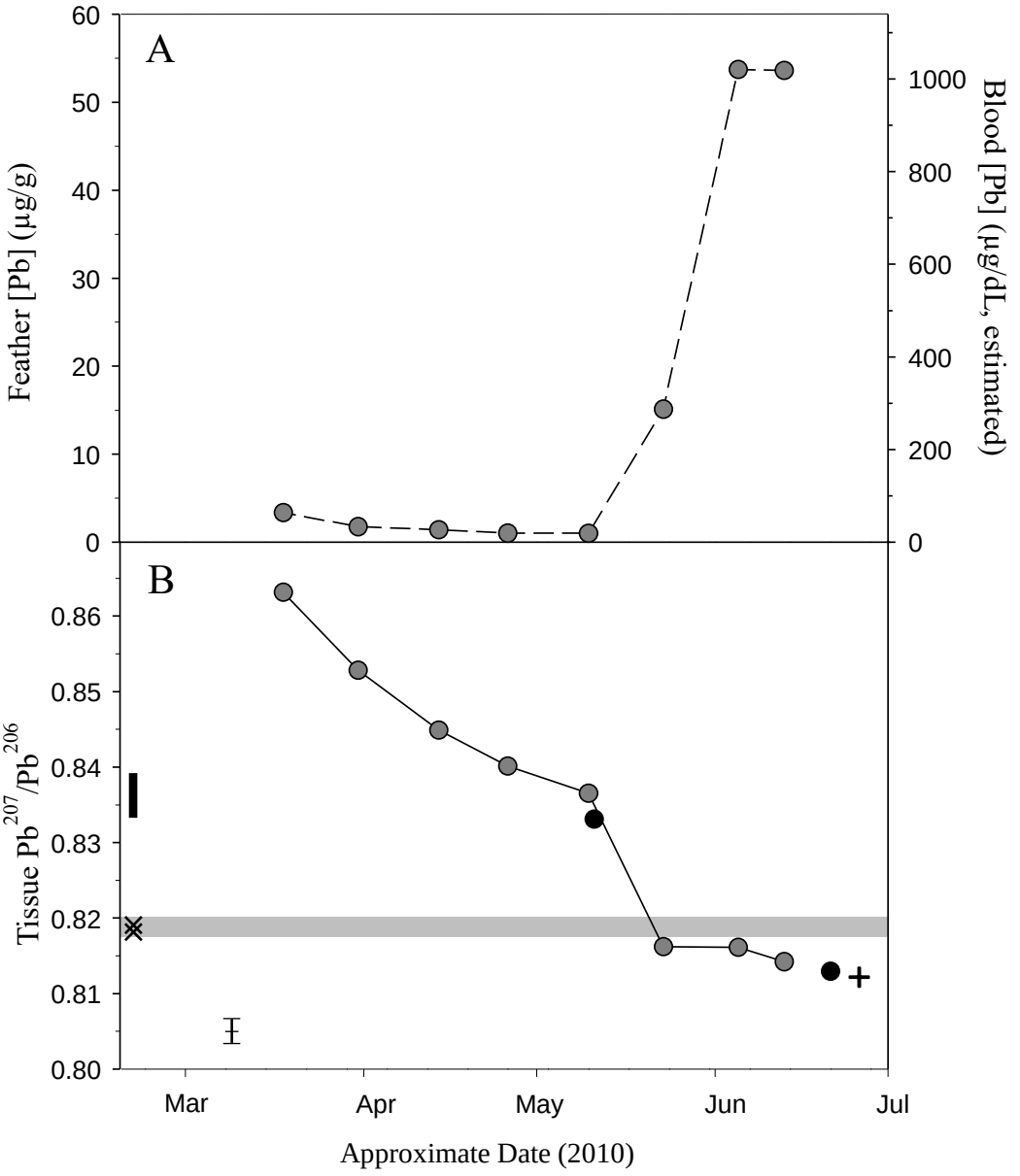
A
B
Feather [Pb] (µg/g)
Blood [Pb] (µg/dL, estimated)
Tissue Pb207/Pb206
Approximate Date (2010)
Mar
Apr
May
Jun
Jul
60
50
40
30
20
10
0
1000
800
600
400
200
0
0.86
0.85
0.84
0.83
0.82
0.81
0.80

Figure 4. Feather lead concentration and isotope ratios surrounding known buckshot ingestion. Panel A) Condor 401 feather lead concentration (left axis) (—●—) versus estimated calendar date. Blood lead concentrations (estimated, right y-axis) calculated from measured feather lead concentrations using a blood (µg/dL):feather (µg/g) lead concentration relationship of 19:1 (Finkelstein et al., 2010). Panel B) Condor 401 feather $^{207}Pb/^{206}Pb$ ratios (—●—) versus estimated calendar date. Also shown are $^{207}Pb/^{206}Pb$ ratios of blood samples (●), embedded birdshot pellet (**X**, right axis) and ingested buckshot (**+**) recovered from condor 401 during treatment for lead poisoning (plotted on collection date). Noteworthy is that 401's oldest (i.e., first-to-grow) feather segment had a $^{207}Pb/^{206}Pb$ ratio of 0.8631 and a lead concentration of 3.36 µg/g (estimated blood lead level ~60 µg/dL); the lead concentration and isotope ratio profiles evidence this bird was recovering from a prior lead exposure event to a source with a $^{207}Pb/^{206}Pb$ signature ≥0.86. In the middle of May 2010, approximately 2 months after the feather started to grow, the feather $^{207}Pb/^{206}Pb$ ratios measurably decline (from 0.8365 to 0.8162), while the feather lead concentrations increase until the last feather segment (from ~1µg/g to ~53.6 µg/g); the $^{207}Pb/^{206}Pb$ ratio in the final, newest-to-grow segment is measurably indistinguishable from the isotope ratios of the blood and ingested buckshot. Black vertical bar on y-axis represents the average ± 2 RSE background lead $^{207}Pb/^{206}Pb$ ratios in pre-release condors in California ($^{207}Pb/^{206}Pb$ = 0.8362 ± 0.0028, n = 22) (Finkelstein et al., 2012). Horizontal shaded bar represents the average $^{207}Pb/^{206}Pb$ value (± 2RSD, n=8) of the recovered embedded birdshot pellets (0.8188 ± 0.0012). The error bar in lower left reflects the $^{207}Pb/^{206}Pb$ measurement error (i.e., 2RSD, see methods). Calendar date estimated from feather length using a primary feather growth rate of 4.4 mm/day (Finkelstein et al., 2010) (Appendix B).

A comparison of the resultant blood lead levels from ingested versus embedded lead shot in condor 401 demonstrates that ingested lead shot produces substantially higher (~30-fold) blood lead levels than tissue-embedded lead shot (i.e., 556 versus 16.6 µg/dL). While many factors can influence the dissolution of lead from embedded shot into surrounding tissues, such as the number, size, and surface area of birdshot versus buckshot pellets acquired by condor 401, the comparison of blood lead values clearly indicates that lead shot ingestion possesses a substantially greater exposure risk compared to embedded shot. This result further supports our conclusion that the severe lead poisoning cases of condors 286 and 375 (blood leads 155 and 180 µg/dL, respectively) are likely due to ingestion of birdshot and not the embedded pellets in their tissues.

4. Conclusion

Our study illustrates the utility of lead isotope analysis of blood and feather tissues to inform circumstances surrounding the illegal shooting of the endangered California condor. Others have used lead isotope analysis in homicide investigations (Gulson et al., 2002; Stupian et al., 2001), yet to our knowledge, we provide the first use of this approach to retrospectively examine the illegal shooting of an endangered species. Our results suggest that lead isotope analysis of tissues and recovered ammunition could be utilized for other wildlife species to aid in the identification of potential commonalities between shooting events. Wildlife rehabilitation centers report gunshot injuries to be responsible for as much as 10% of raptor species morbidity and mortality (Deem et al., 1998; Molina-Lopez et al., 2011), thus additional

information to understand the circumstances surrounding shooting-related injuries might be beneficial for wildlife conservation, law enforcement, and management.

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The use of vertebrate samples for this research was approved by The University of California Santa Cruz's Institutional Animal Care and Use Committee (IACUC).

References

Cade, T. J., 2007. Exposure of California condors to lead from spent ammunition. J.Wildl. Manage. 71, 2125-2133.

California Rules of Court. Uniform Bail and Penalty Schedules. In: Judicial Council of California, (Ed.), Vol. Rule 4.102, July 2011.

Church, M. E., Gwiazda, R., Risebrough, R. W., Sorenson, K., Chamberlain, C. P., Farry, S., et al., 2006. Ammunition is the principal source of lead accumulated by California Condors re-introduced to the wild. Environ. Sci. Technol. 40, 6143-6150.

Deem, S. L., Terrell, S. P., Forrester, D. J., 1998. A retrospective study of morbidity and mortality of raptors in Florida: 1988-1994. J. Zoo Wildl. Med. 29, 160-164.

Farrell, S. E., Vandevander, P., Schoffstall, J. M., Lee, D. C., 1999. Blood lead levels in emergency department patients with retained lead bullets and shrapnel. Acad. Emerg. Med. 6, 208-212.

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

Finkelstein, M. E., George, D., Scherbinski, S., Gwiazda, R., Johnson, M., Burnett, J., et al., 2010. Feather lead concentrations and Pb-207/Pb-206 ratios reveal lead exposure history of California condors (*Gymnogyps californianus*). Environ. Sci. Technol. 44, 2639-2647.

Finkelstein, M. E., Gwiazda, R. H., Smith, D. R., 2003. Lead poisoning of seabirds: Environmental risks from leaded paint at a decommissioned military base. Environ. Sci. Technol. 37, 3256-3260.

Gulson, B. L., Eames, J. C., Davis, J. D., 2002. Evaluation of exhibits from a murder case using the lead isotope method and scanning electron microscopy. J. Forensic Sci. 47, 1015-1021.

Gwiazda, R., Campbell, C., Smith, D., 2005. A noninvasive isotopic approach to estimate the bone lead contribution to blood in children: Implications for assessing the efficacy of lead abatement. Environ. Health Perspect. 113, 104-110.

Gwiazda, R., Woolard, D., Smith, D., 1998. Improved lead isotope ratio measurements in environmental and biological samples with a double focusing magnetic sector inductively coupled plasma mass spectrometer (ICP-MS). J. Anal. At. Spectrom. 13, 1233-1238.

Gwiazda, R. H., Smith, D. R., 2000. Lead isotopes as a supplementary tool in the routine evaluation of household lead hazards. Environ. Health Perspect. 108, 1091-1097.

Molina-Lopez, R. A., Casal, J., Darwich, L., 2011. Causes of morbidity in wild raptor populations admitted at a wildlife rehabilitation centre in Spain from 1995-2007: A long term retrospective study. PLoS ONE 6(9): e24603. doi:10.1371/journal.pone.0024603.

Outridge, P. M., Evans, R. D., Wagemann, R., Stewart, R. E. A., 1997. Historical trends of heavy metals and stable lead isotopes in beluga (*Delphinapterus leucas*) and walrus (*Odobenus rosmarus rosmarus*) in the Canadian Arctic. Sci. Total Environ. 203, 209-219.

Parish, C. N., Hunt, W. G., Feltes, E., Sieg, R., Orr, K., Lead exposure among a reintroduced population of California Condors in northern Arizona and southern Utah. In: R. T. Watson, et al., Eds.), Ingestion of Lead from Spent Ammunition: Implications for Wildlife and Humans. The Peregrine Fund, Boise, Idaho, USA, 2009, pp. 259-264.

Rideout, B. A., Stalis, I., Papendick, R., Pessier, A. P., Puschner, B., Finkelstein, M. E., et al., 2012. Patterns of mortality in free-ranging California condors (*Gymnogyps californianus*). J. Wildl. Dis. 48, 95–112.

Sahagun, L., $40,500 Reward offered in shooting of two condors. Vol. 2013. Los Angeles Times., 2009. <<http://articles.latimes.com/2009/apr/10/local/me-condors-shot10>> accessed Dec, 3, 2012.

Scheuhammer, A. M., Templeton, D. M., 1998. Use of stable isotope ratios to distinguish sources of lead exposure in wild birds. Ecotoxicol. 7, 37-42.

U.S. Fish and Wildlife Service, Endangered Species Act of 1973. In: Dept. of the Interior, (Ed.), Washington D.C., November 24, 2003.

Smith, D. R., Niemeyer, S., Flegal, A. R., 1992. Lead sources to California sea otters: Industrial inputs circumvent natural lead biodepletion mechanisms. Environ. Res. 57, 163-174.

Smith, D. R., Osterloh, J. D., Flegal, A. R., 1996. Use of endogenous, stable lead isotopes to determine release of lead from the skeleton. Environ. Health Perspect. 104, 60-66.

Snyder, N. F. R., Snyder, H. A., 2000. The California Condor: A Saga of Natural History and Conservation. Academ. Press, Lond.

Stupian, G. W., Ives, N. A., Marquez, N., Morgan, B. A., 2001. The application of lead isotope analysis to bullet individualization in two homicides. J. Forensic Sci. 46, 1342-1351.

Sturges, W. T., Barrie, L. A., 1987. Lead-206-207 isotope ratios in the atmosphere of North America as tracers of USA and Canadian emissions. Nature (Lond.). 329, 144-146.

Walters, J. R., Derrickson, S. R., Fry, D. M., Haig, S. M., Marzluff, J. M., Wunderle, J. M., 2010. Status of the California Condor (*Gymnogyps Californianus*) and efforts to achieve its recovery. Auk. 127, 969-1001.

CHAPTER 3: ASSESMENT OF GLUCOCORTICOIDS IN CALIFORNIA CONDOR PLASMA, URATES AND FEATHERS

Zeka E. Glucs, Donald R. Smith, Christopher W. Tubbs, Jennie Jones Scherbinski, Alacia Welch, Joseph Burnett, Michael Clark, Curtis Eng, Myra Finkelstein

Abstract

Vertebrates respond to stressful stimuli with the secretion of glucocorticoid (GC) hormones, such as corticosterone (CORT), and measurements of these hormones in wild species can provide insight into physiological responses to environmental and human-induced stressors. California condors (*Gymnogyps californianus*) are a critically endangered and intensively managed avian species for which information on GC response to stress is lacking. Here we evaluated a commercially available I^{125} double antibody radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA) kit for measurement of CORT and GC metabolites (GCM) in California condor plasma, urate, and feather samples. The precision and accuracy of the RIA assay outperformed the ELISA for CORT and GCM measurements, and CORT and GCM values were not comparable between the two assays for any sample type. RIA measurements of total CORT in condor plasma collected from 41 condors within 15 minutes of a handling stressor were highly variable (median: 70 ng/mL, range: 1 – 189 ng/mL) and significantly different between wild and captive condors ($p = 0.02$, two-tailed t-test, n = 10 wild and 11 captive). Urate GCM levels (median= 620 ng/g dry wt., range= 0.74 – 7200 ng/g dry wt., n=216) significantly

increased within 2 hr of the acute handling stressor (p=0.032, n=11, one-tailed paired t-test), while feather section CORT concentrations (median= 18 pg/mm, range=6.3 - 68 ng/g) also varied widely within and between feathers. Comparison of multiple regression linear models shows condor age as a significant predictors of plasma CORT levels, while age, sex, and plasma CORT levels predict GCM levels in urates collected within 30 min of the start of handling. Our findings highlight the need for validation when selecting an immunoassay for use with a new species, and suggest that non-invasively collected urates and feathers hold promise for assessing condor responses to acute or chronic environmental and human-induced stressors.

1. Introduction

When used appropriately, assessment of glucocorticoid (GC) hormone levels in wildlife species can be a meaningful indicator of physiological stress and ability to respond to energetic demands of their environment (Keay et al., 2006; Madliger et al., 2015; Millspaugh and Washburn, 2004; Walker et al., 2005). The time of sample collection in relationship to a known stressor informs interpretation of GC data in wild animals. Measurement of circulating GC levels prior to a stressor has been interpreted as the allostatic load or “baseline” physiological GC requirement(McEwen and Wingfield, 2003; Wingfield and Kitaysky, 2002), and is often subject to seasonal and diel modulation due to predictable differences in energy availability and energy requirements (Jachowski et al., 2015; Landys et al., 2006). Measurements of elevated GC after a known stressor provide information on responsiveness of the GC secretion pathway, which can also be impacted by chronic stress (Busch, 2010). When the experimental stressor is not precisely controlled, individual plasma GC

measurements are difficult to interpret as they represent a single time point within the hormone stress response that typically occurs over hours to days (Wingfield et al., 1999).

In light of these issues, plasma GC measurements are not always appropriate or feasible for GC stress response comparisons in field settings. Methods for GC quantification in other sample types such as feces, urates, fur, and feathers have been developed for assessing stress status in wild animals (Möstl et al., 2005; Romero and Fairhurst, 2016). Measurements of GCs and GC metabolites (GCM) in feather and urates provide a means for assessing baseline or elevated GC levels in wild condors because of the longer time lag between stressor and hormone elevation, as in urates, or the ability to retrospectively assess circulating GC levels integrated over days to months, as in feathers (Sheriff et al., 2011).

The development of enzyme-linked immunosorbent assay (ELISA) kits for hormone detection has made GC measurements more accessible, as ELISA methods obviate the need for specialized laboratory equipment required for measurement of radioactive materials in traditional radioimmunoassay (RIA)-based methods, or the need for radioactive waste disposal (Sheriff et al., 2011). Many immunoassay kits are optimized for detecting parent hormones in mammalian plasma, although manufacturers sometimes advertise kit compatibility with other biological samples such as saliva and excreta (e.g. Corticosterone ELISA, Enzo Life Sciences). Immunoassay kits have been validated for GC and GCM measurement in a variety of non-plasma sample types in birds [in feces 14,15; in feathers 16]. However, metabolic pathways for GCs and sample matrix composition can be both species- and sample-specific, and the antibodies in each immunoassay kit may

interact with sample GC differently depending on GCMs present or matrix composition of the sample (Palme et al., 2005). As such, analytical validation of immunological GC and GCM measurement methods for the specific target species and sample type (e.g., plasma, feces, urates, etc.) is needed before embarking on studies to assess GC and GCM concentrations as an indicator of physiological stress (Möstl et al., 2005; Nakagawa et al., 2003; Sheriff et al., 2011; Touma and Palme, 2005).

The California condor (*Gymnogyps californianus*) is a critically endangered vulture for which no GC measurements have been reported to our knowledge. Wild condors undoubtedly experience a variety of stressors, such as frequent lead poisoning (Finkelstein et al., 2012), and semi-annual capture and handling for health monitoring, tracking equipment maintenance, and clinical intervention for lead poisoning events if warranted (United States Fish and Wildlife Service, 2013). Direct physiological measurements are necessary for assessing individual condor health as the population scale metrics often used for wildlife studies, such as survival and reproductive rates, are manipulated by endangered species management protocols (United States Fish and Wildlife Service, 2013). The natural, lead, and management-related stressors experienced by condors may prove deleterious to their health and survival and underscore the pressing need for assessing California condor GC measurements. Collecting pre-stressor plasma is difficult if not impossible in wild avian species such as the condor that are very large and difficult to trap and handle. The process of capturing condors within a flight pen and performing a blood draw can take tens of minutes, but circulating GCs in other avian species have been found to elevate within 2-3 minutes of a handling stressor (Romero and Reed, 2005).

Therefore, collection of peripheral samples such as urates and feather for GC measurement could enable researchers to assess GC status in condors trapped from the wild.

In this study, we identify an appropriate method to compare the condor GC stress-response among tissues and between individual condors, and investigate the potential influence of biological factors (i.e. age, sex, and season) and existing California condor trapping protocols on GC release in this species. We evaluated the precision and accuracy of a competitive corticosterone (CORT) enzyme-linked immunosorbent assay (ELISA) (Cat. No. ADI-900-097, Enzo Life Sciences), and a corticosterone ^{125}I double antibody radioimmunoassay (RIA) kit (Prod. No. 07120103, MP Biomedicals) for measurement of plasma and feather CORT concentrations and urate GCM concentrations in California condors. The endangered status of the California condor contraindicated pharmacological challenges used in some species to test GC responsivity, such as adrenocorticotropic hormone or dexamethasone injections. In light of this constraint, we performed a biological method validation using handling and restraint as the acute stressor, as recommended by Touma and Palme (Touma and Palme, 2005) and employed in other field studies (Herring et al., 2012). We present our method validation and in depth examination of the factors influencing GC levels as a framework for wildlife biologists preparing to measure and interpret GCs in a previously unstudied, free-ranging species, or hoping to use a single immunoassay for GC measurement across multiple sample types.

2. Materials and methods

2.1 Study subjects

Wild condors (n=41) from central California, managed by Pinnacles National Park and Ventana Wildlife Society, and captive condors (n=11) housed at the Los Angeles Zoo and Botanical Gardens and Santa Barbara Zoo were sampled for plasma and urates between 2013 and 2016, and feathers between 2008 and 2010 (SI Table 1). Both male and female condors were included, with ages ranging 1-36 years (SI Table 1). The use of vertebrate samples for this research was approved by The University of California Santa Cruz's Institutional Animal Care and Use Committee with permission from Los Angeles Zoo and Botanical Gardens and Santa Barbara Zoo (IACUC office approval code FINKM1307). Samples were collected at Pinnacles National Park and Ventana Wildlife Society under USFWS sub-permits (Pinnacles National Park Permit # TE 157291-1; Ventana Wildlife Society Permit # TE-026659-14).

2.2 Stress challenge and sample collections

Plasma, urates, and feather samples were collected during scheduled handling events for routine health monitoring as part of condor management protocols (United States Fish and Wildlife Service, 2013). Wild condors were passively captured using carcass-baited double door traps, operated by technicians concealed within a blind to prevent detection of human presence at the trap. Birds were then given access through another blind-operated door to a larger, netted, flight pen (dimensions ~7.5 m x 12 m x 6 m tall). Birds were typically held in the larger flight pen for >24 hr (range=19-223 hr, median=45 hr) before handling commenced.

Captive birds were housed permanently in flight pens of similar dimensions and design. Condors in flight pens were normally housed in groups of 2-9 individuals and had access to food and water *ad libitum*, or in the case of captive individuals, access to food on a weekly basis with set fast days to mimic condor feeding behavior in the wild. While in the flight pen, condors were visually isolated from humans until the day of handling.

On the day of handling, the time of initial pen entry by technicians was recorded for each handling event (median=61 min, range=10-200). Following pen entry by technicians, condors were herded either onto the ground or into enclosed isolation pens, a process that can take 2-15 min. Handling start was recorded upon a bird's capture by a large, hand-held hoop net. The condor was then restrained in hand for a median time of 27 min (range=14 - 47 min) for blood draw, feather collection, physical examinations, and in the case of wild birds, tracking equipment maintenance. Information on body condition (keel ratings and hydration status) were collected for some but not all individuals during these exams (SI Table 1). Keel ratings were collected by feel and hydration status was collected by visual observation of skin elasticity after pinching, and therefor both were subject to variation based on technician. To minimize technician-related bias, we coded these categorical observations as binary (Keel status: 0= breast muscle concave to keel, 1= breast muscle even with or convex to keel; Hydration status: 0= dehydrated, 1=well hydrated).

2.2.1 Plasma collection

Blood samples were collected within 3-18 min of handling start (median= 6 min). Blood (1 – 2 mL) was collected from the metatarsal vein into heparinized BD

Vacutainers® and placed on ice. Plasma was separated from whole blood via centrifugation (10 min at 2,000 x g) within 12 hr of blood collection, transferred into cryovials, and stored at -80˚C until analysis.

2.2.2. Urate collection

Immediately after handling for routine health monitoring as described above, condors were placed into a modified dog kennel similar to those used for condor transport, but altered with a raised vinyl-coated mesh floor above a removable Plexiglas tray to collect urate and fecal samples. The environment inside the kennel was low light and visually isolated from outside stimuli. The kennel had sufficient room to allow the condor to turn around and lay in repose. Wild birds were held within kennels for 1-6 hr before release to the wild or transport to clinics for lead poisoning or other medical treatment if warranted, which was as long as was logistically feasible. Condors did not have access to food and water while in the kennel. Urate and fecal samples were serially collected from the sub-floor tray which was checked at 15 min intervals while birds were kenneled. However, if urate excretion was detected (by sound) before the 15 min tray check, it was collected immediately. Urates were placed on dry ice within 30 min of defecation and stored at -80˚C until extraction to arrest hormone metabolism by bacterial enzymes (Möstl et al., 2005; Touma and Palme, 2005).

As with other new world vulture species, California condors perform urohidrosis, and generally excrete fecal (i.e. solid) and urate (i.e. liquid) material separately (Finkelstein et al., 2015). Since urates were collected more consistently and frequently during condor kenneling time periods, we chose to use GCM levels in urates for comparison across individuals in this study. In the few cases when urates

and feces were excreted together, we collected the dark solid excrement and white-clear liquid urate excrement separately. In order to examine if there were differences across urate samples with respect to hydration and potential fecal mixing, we recorded a color code of 1-5 for each urate sample collected (1=white/clear, 2=white/yellow, 3=yellow, 4=some green, 5=green/brown).

2.2.3. Feather collection

We utilized previously collected feather samples, as the complete trailing edge of flight feather vane (base to tip) is regularly collected during handling events as part of lead exposure monitoring studies [23–25]. Feather vane material from growing and nearly full-grown flight feathers was either collected at the time of handling, or if only partially grown, flight feathers were marked via notching, measured from the base of the feather (at skin level) to tip, and later sampled at a subsequent handling event when the feather was fully grown (SI Table 2). Feather samples were stored at room temperature in plastic bags in low light.

2.3. Sample processing and hormone extraction

2.3.1. Plasma processing

Plasma samples were diluted and analyzed in accordance with both kit instructions with the exception of using half volumes of all samples and kit reagents in the MP Biomedicals radioimmunoassay as described and validated for avian plasma in Washburn et al. (Washburn et al., 2002).

2.3.2. Urates processing and extraction

Whole urate samples were lyophilized and re-suspended in 80% methanol (Certified ACS, A412-4, Fisher Scientific) at a ratio of 75 mL:1 g dry weight; because of differences in urate sample weights, a range of 1-32 mL methanol was added per

sample (adapted from methods (Herring and Gawlik, 2009; Rettenbacher et al., 2004; Tempel and Gutiérrez, 2004)). The sample-solvent mixture was vortexed for 15 sec, shaken at room temperature for 30 min on an orbital table top shaker, and then centrifuged at 2,500 x g for 15 min. Methanol supernatant was transferred to a new vial, evaporated to dryness under vacuum or nitrogen at room temperature, and stored at -80˚C until analysis. Extraction efficiency of a CORT standard (Enzo Life Sciences, NY) was 98% (SI Table 3).

2.3.3. Feather processing and extraction

Feathers were visually inspected for external urate contamination and where found it was removed with water and gentle wiping. Feather vane samples were analyzed in 2 cm sections and corticosterone was extracted based on Bortollotti et al. (Bortolotti et al., 2008), which reported >90% recovery of a radioactive corticosterone spike. Specifically, each feather section was cut into < 5mm pieces with stainless steel scissors and extracted overnight in 10 mL methanol, shaking at 50˚C. Vacuum filtration through 47mm glass microfiber filters (Whatman®, Cat No. 1820 047) was used to remove feather material from the methanol extract. After filtration the extraction vial, filter, funnel, and collection flask were rinsed with ~3 mL additional methanol, which was added to filtered extract. Extracts were then evaporated to dryness under vacuum and stored at -80˚C until analysis.

2.4. Corticosterone and glucocorticoid metabolite measurement methods

As CORT is the primary GC in avian species (Harvey et al., 1984), we tested two immunoassays that were optimized to measure CORT in rodent plasma: a competitive CORT enzyme-linked immunosorbent assay (ELISA) (Cat. No. ADI-900-097, Enzo Life Sciences) and a CORT I^{125} double antibody radioimmunoassay (RIA)

kit (Prod. No. 07120103, MP Biomedicals). The manufacturers' protocols were followed, with samples being re-suspended and diluted in the respective kit's buffers and analyzed in accordance to the manufacturer's instructions, with the exception of using half volumes of all kit reagents in the MP Biomedicals radioimmunoassay as described in Washburn et al. (Washburn et al., 2002).

An 'analytical consistency' sample was generated by pooling aliquots of condor plasma samples together, thoroughly mixing, sub-aliquoting into vials and storing at -80°C; an aliquot of this consistency plasma sample was included in each assay to assess within and between assay reproducibility. To test RIA interassay precision across sample types, a pooled feather extract was analyzed across assays as noted for plasma above, and nine urate samples were run on two different kits to provide a percent difference measurement (% diff) for between assay reproducibility for each kit. Standard curves for each assay day were generated by fitting measurements from serially diluted CORT standards to a 4-parameter logistic curve; CORT and GCM concentrations in samples were calculated from these standard curves. Reported non-target compound cross-reactivities for the ELISA kit antibody were 28% for deoxycorticosterone, 1.7% for progesterone, and < 1% for all other steroids tested by the manufacturer (ELISA kit manual, Enzo Life Sciences). Cross-reactivities reported for RIA kit antibody were < 1% for all steroids tested, with deoxycorticosterone having the highest tendency for cross reaction at 0.34% (RIA kit manual, MP Biomedical).

2.5. Analytical method validation

To validate the hormone measurement methods we evaluated effects of sample matrix composition, CORT spike recovery, assay reproducibility and

precision for each sample type on both ELISA and RIA (Sheriff et al., 2011; Touma and Palme, 2005). To evaluate the effect of sample matrix composition on GC measurements we i) assessed parallelism of serial dilutions of a composite sample of each sample type (plasma, urate extract, and feather extract) with the standard curve, and ii) compared the measured hormone concentrations of serially diluted samples to their expected concentrations. To calculate the expected concentrations, we began with a sample CORT or GCM concentration from the most dilute extract, and calculated the expected concentration of the rest of the dilutions in the series using the dilution factor. For spike recovery experiments, known volumes of kit CORT standard were added to the plasma (50-460 pg CORT added), urate extract (10 -280 pg CORT added), or feather extracts (30-130 pg CORT added), and analyzed in triplicate with un-spiked aliquots of the same samples for comparison (SI Table 4). Thus, spike recoveries reported for these sample types reflects analytical and not total procedural (i.e. extraction efficiency and analyte quantitation) accuracy. For assay precision, aliquots of pooled samples were analyzed in triplicate within a single assay. Plasma sample aliquots were used to determine inter-assay precision for ELISA and RIA, and urate and feather extracts were analyzed 2-3 times across RIA for further validation.

2.6. Statistical analyses

We used relative standard deviation values to compare precision of the immunoassays tested. Spike recoveries and other mean values are presented as mean ± standard deviation. We calculated the Pearson's R to test for correlation to compare RIA and ELISA GC measurements. We employed a paired t-test for our validation that urate GCM increased significantly within 2 hr of a handling event. For

all statistical tests, a significance level of <0.05 was used for null hypothesis rejection. Data analyses were performed using JMP® Pro (Version *12* SAS Institute Inc., Cary, NC).

To determine which predictor variables influenced condor plasma CORT, or urate GCM levels we used an information theoretic approach (Burnham and Anderson, 2002) and multiple regression models. Values of continuous predictor variables were scaled before regressions by dividing by 2 times the standard deviation, so the magnitude of coefficients could be directly compared (Gelman, 2008). Plasma CORT response variable is reported as nanograms per milliliter (ng/mL) and urate GCM response variable as nanograms per gram dry weight (ng/g dry wt). Feather CORT was excluded from this analysis due to limited sample size.

We identified covariance between predictor variables using Spearman's ρ correlations. We then constructed and ranked *a priori* models using second order Akaike's Information Criterion (AIC_c) to control for small sample sizes. Candidate models for plasma CORT and urate GCM included condor age, sex, season (spring= May 1- Jun 30; fall= Aug 1- Oct 30), keel status, hydration status, and time since stressor parameters (i.e. hours since trapped from wild, minutes since pen entry by technician, minutes since handling start). First urate GCM models also included plasma CORT as a parameter. Information on keel and hydration status was not available for all individuals (SI Table 1). The decision to exclude these predictors from the final set of candidate models for first urate GCM was reached based on a finding of no significance in a prior round AIC_c model selection with the subset of samples for which this information was available and the goal of maximizing sample size for model inference. Both model sets included global models with all variables,

and an intercept only model. The models with the lowest AIC_c value was considered the most parsimonious (Burnham and Anderson, 2002). We calculated differences in AIC_c from the top model (ΔAIC_c) and AIC_c weights for the subset of models that made up 90% of AIC_c weights of the complete model set. These candidate models were then used to infer the influence of each parameter. For both sets of models we summed the Akaike weights for each parameter of influence, calculated their model averaged beta coefficients to estimate the direction and strength of their effect on GC, and determined whether parameters were informative with 90% confidence intervals.

3. Results and Discussion

3.1. Immunoassay comparison and validation

3.1.1. Precision, accuracy, and matrix effect on corticosterone and glucocorticoid metabolite measurements

To evaluate the analytical precision of the ELISA and RIA assays, plasma, urate, and feather samples were analyzed repeatedly within a single analytical run (intra-assay precision; n=3-8 sample replicates run in duplicate) and across multiple analytical runs (inter-assay precision; n=2-5 sample replicates run in duplicate). Generally, the ELISA and RIA assays yielded similarly acceptable intra-assay precision of the plasma CORT, feather CORT, and urate GCM measurements (Table 1). The intra-assay measurement precision ranged from 1–7% relative standard deviation (RSD) for both methods for all sample types, while the inter-assay precision for plasma was 5-8% RSD. The inter-assay precision for pooled feather extracts analyzed across five assays yielded 10% RSD and nine pooled urate

extracts analyzed across two assays produced an average percent difference of 6.8% (range: 1.2-14%). To assess analytical accuracy for each method, plasma, urate, and feather samples were spiked with a known amount of CORT approximately equal to the inherent sample CORT or GCM concentration (based on previous analysis or average values for sample type) before analysis, and the spike recovery was determined (SI Table 4). Overall, CORT spike recoveries were ~100% (± ~1 - 5% SD), with several exceptions (Table 1): the CORT spike in urates was consistently under recovered as measured by ELISA (87 ± 3.0 %), while RIA somewhat over-measured the CORT spike in urates (109 ± 0.8 %). Also, CORT spike recovery in feather samples as measured by ELISA was more variable (119 ± 23 %) than in the RIA (101 ± 4.8 %).

To test for sample matrix interference and determine the range of sample dilutions for which ELISA and RIA assays performed most reliably, parallelism tests were conducted on representative plasma, urate extract, and feather extract by serially diluting samples prior to analyses. In both ELISA and RIA, parallelism tests showed that ‘as analyzed’ concentrations of serially diluted extracts decreased linearly, parallel to the standard curve (SI Fig 1). However, when we converted the ‘as analyzed’ concentrations of these serially diluted samples to ng CORT or GCM/g sample, small differences in ‘as analyzed’ concentrations compounded to create inconsistent concentrations by sample weight (Fig 1). We found measureable and unpredictable effects of sample dilution on GC concentrations by sample weight on

Table 1. Precision and accuracy of enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) measurements of condor plasma, urate extract, and feather extract. All samples were run in duplicate and the number of samples analyzed in is in parentheses.

Method	Sample Type	Intra-assay Precision, %RSD[a]	Inter-assay Precision, %RSD[b] or average %diff*[c]	% Recovery of Corticosterone Spike[d]
ELISA	Plasma	6.6 (3)	7.9 (3)	101 ± 5.6 (3)
	Urates	6.2 (8)	n/a	87 ± 3.0 (5)
	Feather	2.5 (4)	n/a	119 ± 23 (3)
RIA	Plasma	3.2 (3)	4.1 (4)	95 ± 3.6 (3)
	Urates	1.0 (3)	6.8*[c]	109 ± 0.8 (3)
	Feather	3.8 (3)	10 (5)	101 ± 4.8 (4)

[a]Intra-assay precision values are the percent relative standard deviation (%RSD) of sample CORT/GCM measurements analyzed within a single assay run,. Hormone concentrations were normalized to ng CORT/mL plasma, ng CORT/g feather, or ng GCM/g dry weight urate before comparison. [b]Inter-assay precision is reported as %RSD for samples run on three or more assay runs over weeks to months. Samples were aliquotted and stored at -80˚C before kit buffer was added, then diluted in buffer and run on same day. Hormone concentrations were normalized to ng CORT/mL plasma, ng CORT/g feather, or ng GCM/g dry weight urate before comparison. [c]Inter-assay precision is reported as average % difference (%diff) for samples that were run on two assay runs. The %diff value is the average %diff for 9 samples that were run on two different assay runs (range = 1.2 - 14%). These samples were all dissolved in assay buffer and stored at -20˚C between assay runs. [d]The % spike recovery (mean ± standard deviation) reflects the percent of exogenous corticosterone added to sample prior to analysis recovered in assay measurement.

ELISA measurements in all three sample types, with sample concentrations diverging from expected levels in both positive and negative directions (Fig 1 A-C). In contrast, the serially diluted samples analyzed by RIA were generally within the assay measurement error (± 10%) of the expected values, particularly when sample dilutions fell within the range anticipated for typical use of the assay in this study (Fig 1 D-F). Based on these results, the CORT ELISA appears susceptible to sample matrix interferences (shown to both enhance and interfere with signal within a sample type), and thus appears less reliable for urate and feather extract analyses than the RIA.

3.1.2. Poor agreement between enzyme-linked immunoassay and radioimmunoassays for plasma corticosterone, urate glucocorticoid metabolite, and feather corticosterone measurements

Our results show generally poor agreement between the two methods, with the greatest disagreement typically occurring in samples with higher GC or GCM levels (Fig 2). While RIA and ELISA measurements were statistically correlated in plasma (Pearson's $R = 0.65$, $p = 0.04$, $n=10$) and urate samples (Pearson's $R= 0.70$, $p < 0.01$, $n=16$), there was substantial deviation from the expected agreement for many individual samples. There was no significant correlation between GC measurements in feathers (Pearson's $R= 0.61$, $p = 0.11$, $n=8$) (Fig 2).

ELISA measurements for all sample types tended to be lower than RIA measurements. This is most apparent in urate GCM concentrations, where the median ELISA value (28 ng/g) was approximately half of the median RIA value (54 ng/g; see SI Table 5 for information on individual sample origins and dilutions). As

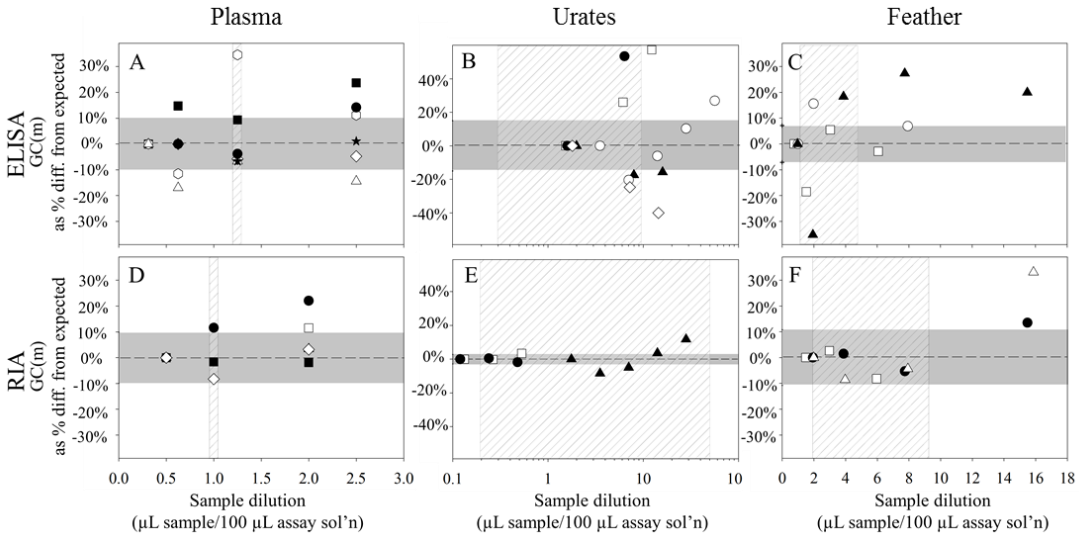

Figure 1. **Plasma corticosterone (CORT) (A and D), urate glucocorticoid metabolites (GCM) (B and E), and feather CORT (C and F) levels measured by radioimmunoassay (RIA) are more reproducible than enzyme-linked immunosorbent assay (ELISA) across a range of sample dilutions.** Symbols (open and filled squares, circles, triangles, and diamonds) represent a sample from an individual condor that was analyzed over a range of sample dilutions. CORT and GCM levels in diluted samples are expressed as a percent difference from expected values (horizontal dashed line at 0% diff.), based on levels measured in the most dilute sample (i.e., the lowest amount of sample in milligrams per 100 µL assay solution), and assumes sample matrix interferences are minimized in this most dilute sample. The vertical hash-marked region in each panel reflects the range of sample dilutions (x-axis) used for all samples in this study. The horizontal grey-shaded region reflects the CORT or GCM measurement uncertainty (± 2 RSD, based on intra-assay precision) for each assay and sample type; symbols within this region do not measurably differ from expected GC levels.

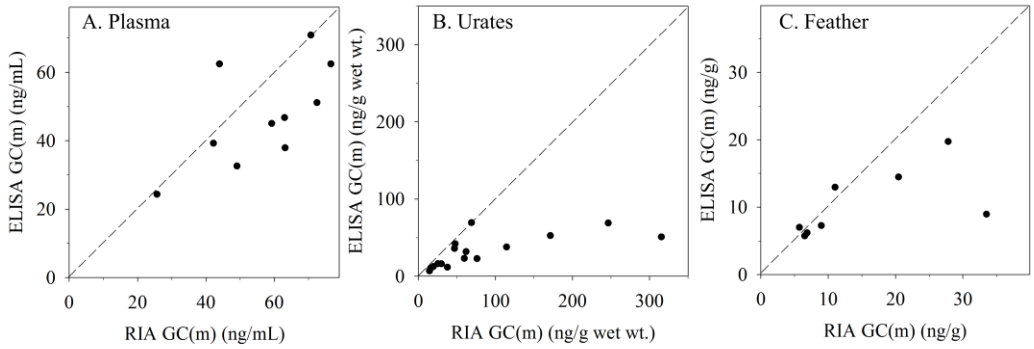

Figure 2. Measurements by radioimmunoassay (RIA) vs. enzyme-linked immunosorbent assay (ELISA) are different but significantly correlated for plasma corticosterone (CORT) and urate glucocorticoid metabolites (GCM). Each data point represents a condor sample measured by both RIA and ELISA. The dashed line indicates idealized agreement (y=x) between the RIA and ELISA values. In all sample types ELISA measurements trended lower than RIA measurements. (A) Plasma CORT concentrations by ELISA and RIA. (B) Urate GCM concentrations measured by RIA and ELISA, levels measured by ELISA are systematically lower by ~50%-600% compared to RIA. (C) Feather CORT concentrations measured by RIA and ELISA appear to agree for lower CORT concentration samples.]

the ELISA yielded urate GCM values both higher and lower than expected for the less dilute samples (Fig 1), the generally lower GCM levels measured by ELISA versus RIA (Fig 2) cannot be attributed to matrix interferences alone.

Our results suggest the ELISA kit antibody reacted with fewer metabolites, or less strongly with the metabolites compared to the RIA kit antibody. Avian plasma contains CORT in its original, secreted form, and feathers are also expected to contain predominantly CORT in secreted form along with some glucuronidated and sulfonated metabolites (Bortolotti et al., 2008). However, urates and feces may contain predominantly metabolized GCs with relatively little parent compound

(Touma and Palme, 2005), and method-based differences in our GC measurements are most notable in condor urate GCM (Fig 2B). Differences in assay performance in urate extracts may be due to the different antibodies utilized in these two kits, which may have differential binding affinity with the GCMs present in condor urate samples, as has been suggested by other immunoassay comparison studies (Warnken et al., 2016). Another possibility is that matrix compounds present in condor urate and feather extracts reduce measurement accuracy by either cross-reaction with the anti-CORT antibodies, or interfering with hormone-antibody binding, as has been found with immunoassay kits and human saliva (Miller et al., 2013; Raff et al., 2002).

While these data do not allow us to determine outright which method is more accurate for measurement of GCs in condor samples, the superior performance of RIA across the sample dilution range suggests that RIA is more reliable than ELISA for comparing GC concentrations in condor samples.

3.2. Handling stress challenge using radioimmunoassay to measure urate glucocorticoid metabolites and feather corticosterone

RIA's superior reproducibility over the sample dilution ranges for urates and feather, and the generally poor agreement between the two methods, with the ELISA typically yielding much lower CORT/GCM concentrations compared to the RIA, led us to select the RIA for the handling stress challenge phase of the study. RIA has also been found to be reliable across several mammalian and avian species (Wasser et al., 2000).

3.2.1. Urate glucocorticoid metabolite concentration increases in response to a stress challenge

We collected sequential urate samples for 2-6 hr following a handling event for a subset (n= 24 condors) of the condors sampled for plasma CORT. We found GCM concentrations were generally stable in fresh urates for at least 30 min following collection and before freezing, similar to findings by Khan et al. (Khan et al., 2002) (SI Fig 2). All urate samples had detectable GCM levels (median= 710 ng/g dry wt., range= 0.74 – 7200 ng/g dry wt., n=216 samples; SI Table 7). Using urate sample wet weight as a proxy for volume, wet weight of urate samples in general decreased over time since handling (Spearman's $\rho = -0.19$, $p= 0.006$, $n= 216$) and urate GCM concentrations on a wet weight basis were negatively correlated with sample wet weight (Spearman's $\rho = 0.02$, $p = 0.75$, $n= 216$), suggesting that GCM wet weight concentrations are influenced by volume (SI Fig 3A and 3C) and the excreted volume of urates may change depending on the hydration state of the bird and more (or less) fluid is reabsorbed in the lower gastrointestinal tract (Braun, 2014). Conversely, urate dry weight did not significantly decrease with time (Spearman's $\rho = 0.02$, $p = 0.75$, $n= 216$, SI Fig 3B). Additionally, urate samples of different colors (coded 1-5) had significantly different wet weight GCM concentrations ($p=0.03$, $n=216$, one-way ANOVA, SI Fig 3D), an indication of hydration state and potential fecal contamination, whereas dry weight urate GCM levels were not significantly affected by the variable of urate color ($p=0.11$, $n=216$, one-way ANOVA, SI Fig 3E). Thus, we present urate GCM levels on a dry weight basis.

To measure the urate GCM response to a handling event we identified the peak GCM value within 2 hours of handling start for the 11 birds handled and kenneled for ≥2 hours. The handling stressor resulted in a significant increase in urate GCM levels over the 2 hours compared to the first urate sample collected

(p=0.032, n=11, one-tailed paired t-test). Moreover, for most birds urate GCM levels remained elevated over the 3.5 hour maximum time in the kennel, with levels appearing to decline and return towards initial (i.e., first sample) GCM concentrations near the end of kenneling in some cases (n= 7, Fig 3). Hirschenhauser et al. (Hirschenhauser et al., 2012) similarly reported for other avian species that after a stressor, urate GCM concentrations remained elevated for several hours. We also found that GCM levels increased ~30-40 minutes post stressor, which is comparable with Legagneux et al.'s (Legagneux et al., 2011) finding that GCM concentrations of snow geese (*Chen caerulescens atlantica*) feces and urates combined collected 40 min after cannon netting were significantly elevated above GCM levels in samples from undisturbed birds. Similarly, Hirschenhauser et al. (Hirschenhauser et al., 2012) observed ~30 minute time lags for GCM increases in serially excreted fecal and urate samples after intravenous radiolabeled CORT injections in quail (*Coturnix japonica*) and chickens (*Gallus domesticus*). Noteworthy is that differences in elapsed time since condors were trapped from the wild or since initial pen entry by a technician before the handling stressor and first urate sample was collected could also be influencing GCM levels reported in the present study, which we investigate with AIC in the following section (see *Factors influencing plasma CORT and urate GCM concentration in California condors*).

We found that the overall magnitude and pattern of change in urate GCM levels following the acute handling stressor varied widely between condors (Fig 3). In some birds, urate GCM levels increased rapidly and by several orders of magnitude (e.g., condors 631, 470, both wild), while in others the increase was slower (e.g., condors 23, 120, 159, 174, 448, 464), with the remaining three birds being somewhat

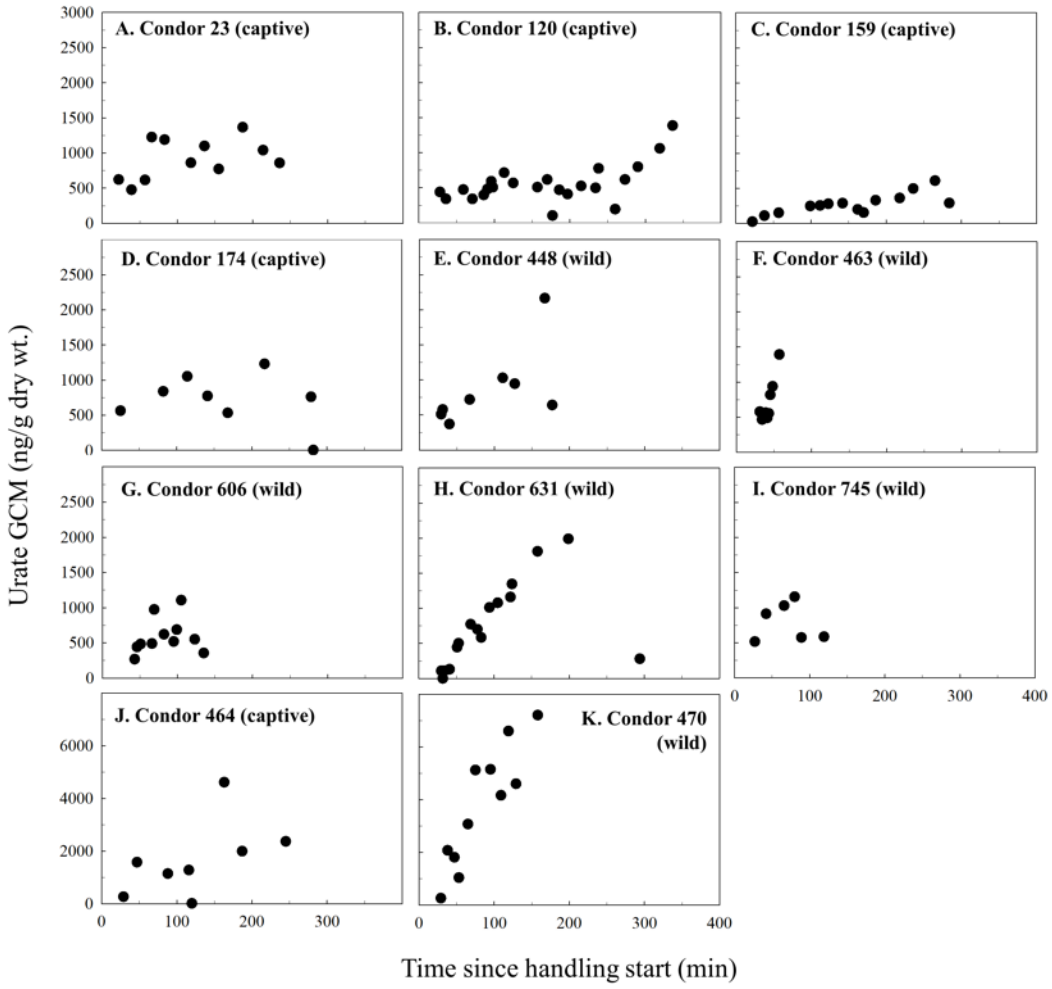

Figure 3. Condor urate glucocorticoid metabolite (GCM) concentrations significantly increased within 2 hours of a handling stressor. GCM concentrations (ng/g dry wt.) in condor urates collected sequentially following a physical handling and venipuncture event. Panels A-D and J are zoo-captive condors, while panels E-I and K are wild condors. X-axis shows the elapsed time since handling start; note y-axis scale difference between condor panels A-L versus J and K. See SI Table 7 for additional information on individual urate samples.

intermediate between these two groups. Other avian species have also shown significant inter-individual variation in the magnitude of GCM responses to a common stressor, as measured in feces of harlequin ducks (*Histrionicus histrionicus*)(Nilsson et al., 2008) and greater sage grouse (*Centrocercus urophasianus*)(Jankowski et al., 2009). While the underlying basis for these individual differences in the pattern/magnitude increase in urate GCM levels is not clear, it is clear that the sequential collection of urates reflects the acute stress of physical handling experienced by these condors and thus may serve as a means to monitor changes in circulating CORT levels in response to a stressor.

3.2.2. Feather corticosterone concentrations vary over time of feather growth

To determine whether feather CORT concentrations vary over the period of feather growth (months) and respond to acute stressor handling events in wild condors, we measured CORT extracted from sections of five flight feathers (four primaries, and one retrix) from five individual condors. For 1 condor (#631), 21 separate ~2 cm sections along entire length of the primary feather were analyzed. For the other four condors 3-5 ~2 cm feather sections per feather were analyzed. All 37 feather sections analyzed had measurable CORT levels (normalized to feather weight, median = 11 ng/g, range = 4.2 - 69 ng/g dry wt.; normalized to feather section length along the rachis, median = 18 pg/mm, range = 6.3 – 68 pg/mm, SI Table 2), and were within the general range of feather CORT levels reported for other avian species(e.g. ~2-50 pg/mm, Bortolotti et al. (Bortolotti et al., 2009); ~4-370 ng/g Koren et al. (Koren et al., 2012a)). To compare feather CORT levels in discrete feather sections over the period of feather growth, we normalized feather CORT

levels to feather section length (mm along the rachis), since Bortolotti et al. (Bortolotti et al., 2009, 2008) and Romero and Fairhurst (Romero and Fairhurst, 2016) provide well-supported cases for normalizing CORT levels to feather section length along the rachis when sections of feathers are analyzed separately. We found that the mass of 2 cm feather sections of California condor primary feathers varied over the length of the feather, and hence the duration of feather growth, due to the feather's tapered shape (SI Fig 4). Feather section CORT concentrations differed depending on whether CORT levels were normalized to feather section mass or length (along the rachis), but we did not observe the decreasing trend in ng/g feather CORT over feather growth reported by Bortolotti et al. (Bortolotti et al., 2009)(SI Fig 5).

Feather section CORT levels (pg CORT/mm feather) varied up to 6-fold within a feather over the ~3 month period of feather growth (Fig 4 A-E). For condor 631 for who the entire primary flight feather was analyzed, there is a clear pattern of increasing then decreasing feather CORT levels over the nearly 4 month period of feather growth (Fig 4; assumes a feather growth rate of 4.4 mm/d (Finkelstein et al., 2010)). The other feathers also showed different patterns of feather section CORT levels, with some (e.g., condor 192, 401) being relatively invariant over the ~30 – 50 day period of feather growth, while others (condors 312, 336) varied more markedly between sections (Fig 4). Many factors may influence CORT deposition into growing feathers, including CORT release into the circulation, feather structure and growth rate, the bird's age, breeding status, and environmental stressors (Romero and Fairhurst, 2016). These factors undoubtedly affect feather CORT levels in California condors, who also may experience acute or chronic stress associated with semi-

annual trapping and handling for lead exposure monitoring, and prolonged clinical treatment if indicated by lead poisoning.

Because California condors are closely monitored and growing feathers often identified and marked when birds are in-hand during routine trap-ups, we were able to estimate the approximate timing of these acute stressor trapping and handling events over the period of feather growth. The increases in feather section CORT levels for these five condors generally, but not exclusively, coincides with the estimated timing of the trapping and handling event (Fig 4), though the coincidence is more apparent for some cases (e.g., condors 631, 192, 336) than others (condors 312, 401). Feather CORT levels have been shown by others to reflect stress-induced increases in CORT over feather growth (Bortolotti et al., 2008). The markedly different patterns in feather CORT levels in these five condors may reflect that the relationship between stress-induced increases in plasma CORT and feather CORT levels varies depending on the nature of the stressor, and that the stress response induced from a ~30 min handling event is too fleeting for some condors to be captured in a 2 cm feather section representing ~4-5 days of feather growth and CORT incorporation.

California condors are regularly lead poisoned (Finkelstein et al., 2012), and lead poisoning has been shown to influence the stress response and circulating CORT levels in birds and mammals (Baos et al., 2006; Virgolini et al., 2005). Here, we measured feather CORT levels from condors for whom lead poisoning status at the time of feather collection was known. For Condors 192, 312, and 401 (Fig 4 B-D),

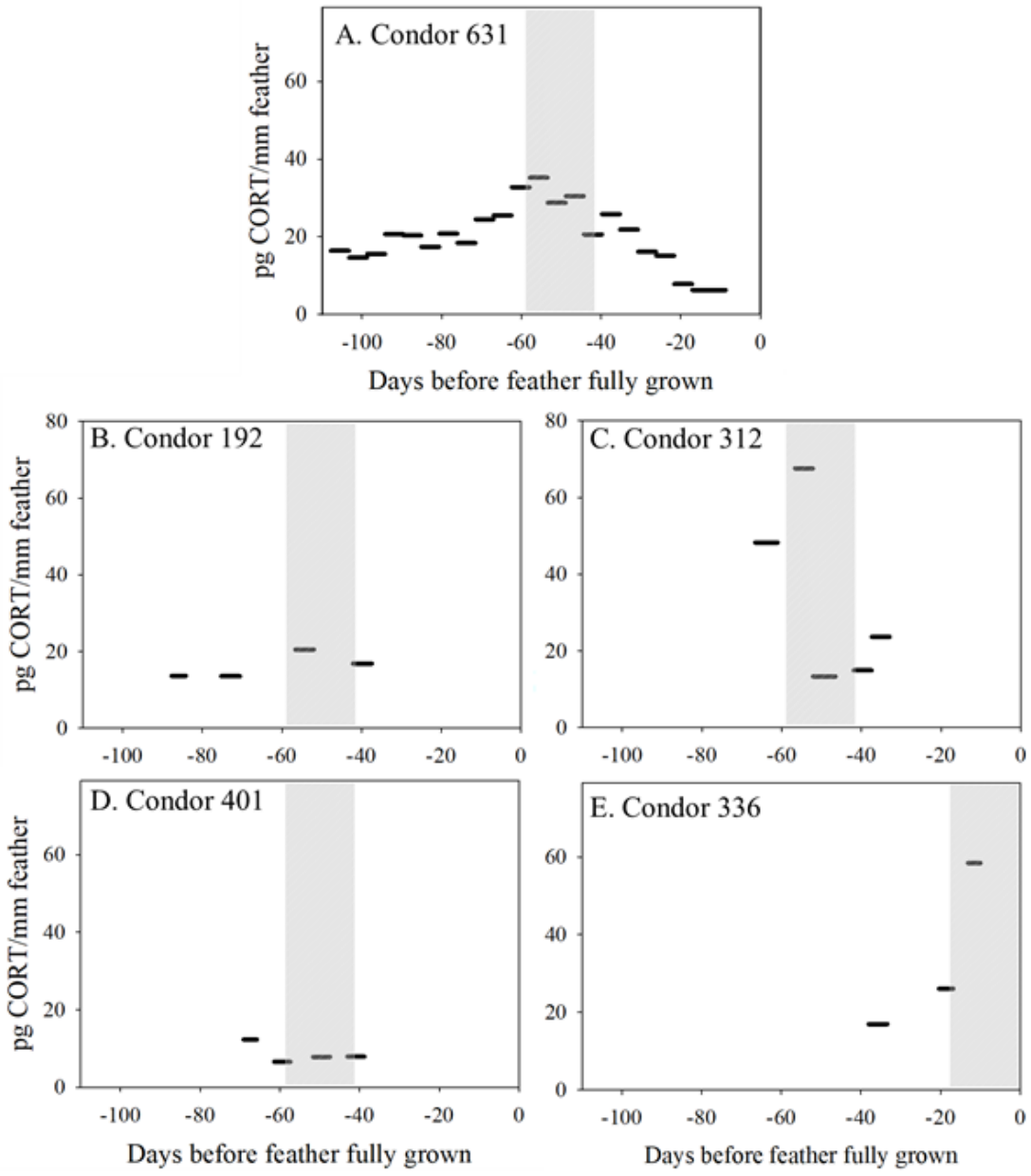
A. Condor 631
B. Condor 192
C. Condor 312
D. Condor 401
E. Condor 336
pg CORT/mm feather
Days before feather fully grown
0
20
40
60
80
-100
-80
-60
-40
-20

Figure 4. Feather section corticosterone (CORT) concentrations vary over the period of feather growth in free-flying California condors. CORT concentrations for sections of flight feather (2 cm lengths along rachis axis) collected from five individual wild condors.

(A**)** Contiguous 2 cm sections from condor 631's primary feather show changes in CORT concentrations over the time of feather growth. **(**B-D**)** Non-contiguous primary feather sections from condors 192, 312, and 401 show changes in feather CORT concentration over time of feather growth and variation between birds. (E) Retrix (tail) feather CORT levels from condor 336, who died of lead poisoning while receiving clinical treatment (feather growth day 0). The estimated duration (days) of feather section growth is represented for each section by the width of each section line, determined using a primary feather growth rate of 4.4 ± 0.28 mm/day in California condors (Finkelstein et al., 2010)(See SI Table 2 for details). Total CORT per feather section (pg) is normalized to feather section length (mm along rachis axis) to represent integrated plasma CORT levels over time of feather section growth (Bortolotti et al., 2009, 2008). Grey shaded area indicates timing of the estimated 18 day period within which the condor was trapped, held captive in a flight pen, and handled.

there was no evidence of elevated lead exposure at the time of feather collection (blood leads <10 ug/dL), and general body condition was normal (data not shown). Thus, variation in feather CORT levels in these three birds is unlikely to have been influenced by lead poisoning. In contrast, Condor 336's feather grew during a known lead poisoning event that ultimately led to the bird's death. Finkelstein et al. (Finkelstein et al., 2010) estimated that condor 336 was lead exposed approximately 75 days before the growing feather was collected (i.e., around feather growth day -75, Fig 4E), and that the estimated peak blood lead level of ~1100 µg/dL occurred ~45 days before the feather was collected (i.e., at ~day -45, Fig 4E), weeks before the bird was found moribund, captured, and transported to the clinic for treatment where it died soon thereafter (Finkelstein et al., 2010). While only a limited number of feather sections were available for CORT analyses, they clearly show a marked increase in CORT levels that coincides with the onset of acute morbidity, capture, and clinical treatment for lead poisoning (Fig 5E). Collectively, these findings suggest that CORT feather levels hold promise as a biomarker for physiological status and/or handling-induced stress in condors, as has been established experimentally for red legged partridges (*Alectoris rufa*) (Bortolotti et al., 2008).

3.3. Factors influencing plasma corticosterone and urate glucocorticoid metabolite concentrations in California condors

3.3.1. Plasma corticosterone

To investigate the effects of trapping and handling stress-related factors (e.g., time since start of handling) and non-handling related factors (e.g., age, sex, body condition, season) on plasma CORT levels, we constructed and ranked *a priori* multiple linear regression models for their likelihood of predicting total (bound and

unbound) plasma CORT levels. Within-species variation in CORT responses to stress is known to be influenced by many factors, including but not limited to sex, age, breeding status, season, and time of day (Wingfield et al., 1999). The total plasma CORT level measured here does not account for differing levels of corticosteroid-binding globulin (CBG) in plasma samples, but is a metric of the CORT reservoir in circulation and quantifies total biological impact of stressors (Schoech et al., 2013). All 52 plasma samples analyzed from 41 individual condors had measurable CORT concentrations (range: 1 – 189 ng/mL, median: 70 ng/mL; SI Table 6). For individuals with multiple plasma samples collected (n=8), one plasma sample per bird was randomly selected to be included in all statistical comparisons including the linear regression models for AIC ranking. There was a significant difference between plasma CORT values measured in captive (55 ± 31 ng/mL, n=11 samples) compared to wild (85 ± 43 ng/mL, n=30 samples) condors ($p=0.02$, n=41, two-tailed t-test, SI Fig 5). The reason for this difference is not clear, but may be due in part to different life histories and trapping procedures for captive vs. wild condors; wild condors are lured into and trapped in the flight pen, then captured and handled for blood draw, etc., whereas captive condors are already fully acclimated to their flight pen and are simply captured and handled for their routine health checks. Given the difference between wild and captive condor plasma CORT levels, we chose to exclude captive condors from our model selection process presented below.

In light of studies in avian species showing that plasma CORT levels begin to elevate above pre-stress baseline within 2-3 minutes of a handling stressor (Romero and Reed, 2005; Schoech et al., 1999), the protocols for capturing the endangered condor from the wild make it difficult, if not impossible, to collect a plasma sample

that represents a true physiological baseline for circulating CORT levels. In the present study, condor blood collection occurred 3-18 minutes since handling start (median = 6 min), and 10-200 min after initial flight pen entry by a technician (median = 61 min), and 1-10 days since the birds were trapped in the flight pen from the wild (median= 45 hr, range = 19-223 hr) (SI Table 6). In light of this, we tested how plasma CORT levels varied in response to several time points in the trapping/handling process. Since multiple wild condors are typically trapped and held in a flight pen at once, birds captured and handled later in the day experienced a greater elapsed time since the initial (for the day) pen entry by a technician, as well as a greater number of technician pen entries before being captured and handled compared to birds that were captured/handled earlier in the day. Thus, as expected, time since initial pen entry by a technician was highly correlated with the time of day of plasma collection (Spearman's $\rho = 0.63$, $p < 0.001$, $n=30$), so we included elapsed time since initial pen entry in the model, but not time of day of plasma collection. Given this, it is possible that the effect of elapsed time since initial pen entry is confounded by a diurnal effect of time of day on plasma CORT levels.

The top two models (≤1 ΔAIC units) explaining variance in plasma CORT levels included the variables age and season (Table 2). These models of age and age + season were 2.03-times more likely than the next best model that included keel status in explaining variance in plasma CORT and explained 32 – 37% of the variance in plasma CORT levels (Table 2). Lower ranked models that included age plus other variables (e.g., keel status, hydration status, minutes since handling start, minutes since initial pen entry, hours since trapped in the flight pen, and sex) performed notably poorer although some models that incorporated more predictor

variables (K= 5-6) explained 1-4% more variance in plasma CORT than the top models (Table 2). Notably, only age appeared to be clearly influential for plasma CORT levels, based on the 90% confidence intervals (CI) for beta coefficients for assessing variable importance in AIC model selection discussed in Arnold (2010); here, only the beta coefficients and CI's for age did not overlap zero, while the others did (Fig 5A; SI Table 8). Consistent with this, we ranked variable weights to assess relative importance in explaining variance in plasma CORT levels (Burnham and Anderson, 2002), and found models containing age had a sum AIC weight of 1.00, followed by season (0.34). The least influential variables were keel status (0.24), hydration status (0.21), minutes since handling start (0.17), sex (0.14), hours since trapped in the flight pen (0.12), and minutes since pen entry (0.11) (SI Table 8). The model-averaged beta coefficient for the significant predictor variables of age is positive, indicating that plasma CORT levels increase with condor age (Fig 5A). We observed no significant influence of sex, season, keel status, or hydrations status on condor plasma GC (Fig 5A).

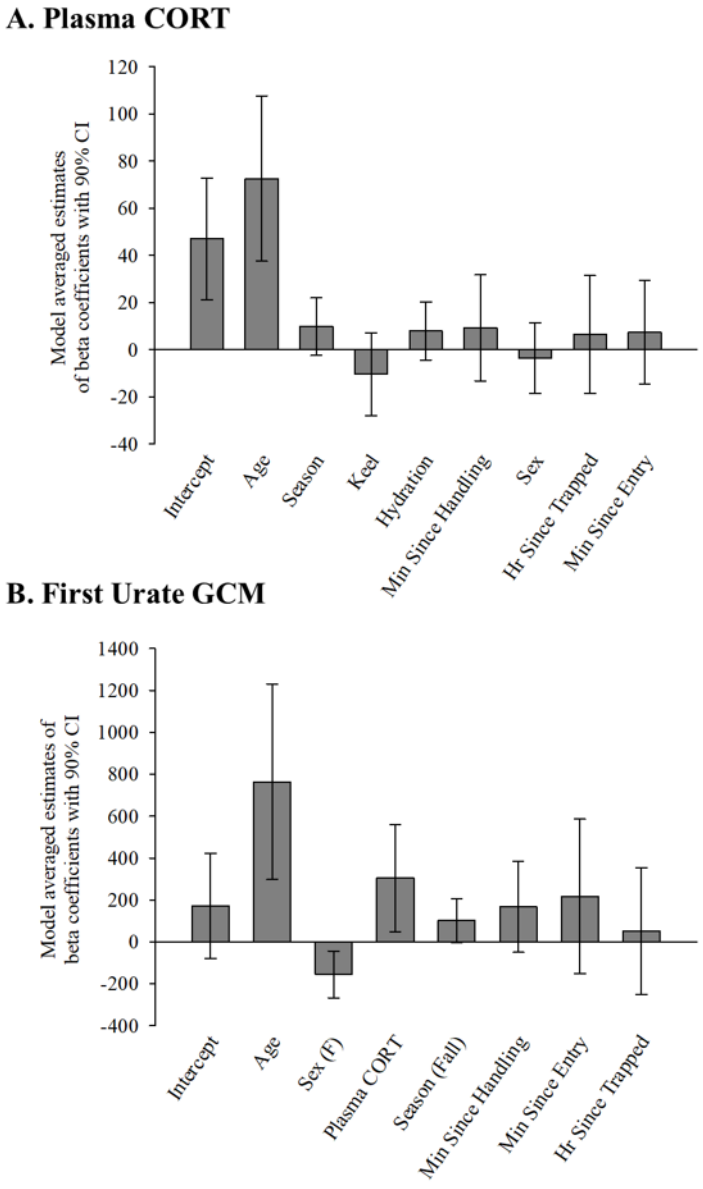

Figure 5. Relative influence of predictor parameters on (A) plasma corticosterone (CORT) values, and (B) urate glucocorticoid metabolite (GCM) values in wild California condors. Model averaged estimate of beta coefficients for all top model parameters with error bars depicting 90% confidence intervals. Parameters with confidence intervals including zero do not have sufficient support for predicting the response variable.

Table 2. Ranking of candidate multiple linear regression models describing variation in plasma corticosterone (CORT) concentrations and glucocorticoid metabolite (GCM) concentration of first collected urates in California condors. Within sample type, the subset of models accounting for 90% of AIC weight and the null model (intercept) are shown.

Model Structure	n	K[a]	-2 Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Evidence ratio[e]	R^2[f]
Plasma CORT								
age	27	3	270.179	277.222	0	0.15	1.00	0.32
age + season	27	4	267.951	277.769	0.547	0.11	1.31	0.37
age + keel	27	4	268.817	278.635	1.413	0.07	2.03	0.35
age + hydration	27	4	268.866	278.684	1.462	0.07	2.08	0.35
age + min since handling	27	4	269.752	279.57	2.348	0.05	3.23	0.33
age + min since entry	27	4	269.953	279.771	2.549	0.04	3.58	0.33
age + sex	27	4	269.987	279.805	2.583	0.04	3.64	0.32
season + hydration + age	27	5	266.988	279.845	2.623	0.04	3.71	0.40
age + hr since trapped	27	4	270.034	279.852	2.630	0.04	3.72	0.32
age + min since entry + season	27	5	267.289	280.146	2.924	0.03	4.31	0.39
age + min since handling + season	27	5	267.333	280.19	2.968	0.03	4.41	0.39
age + keel + hydration	27	5	267.527	280.384	3.162	0.03	4.86	0.38
age + season + keel	27	5	267.581	280.438	3.216	0.03	4.99	0.38
age + sex + season + keel	27	6	264.238	280.438	3.216	0.03	4.99	0.38
age + hr since trapped + season	27	5	267.861	280.718	3.496	0.03	5.74	0.38
age + sex + season	27	5	267.891	280.748	3.526	0.03	5.83	0.38
age + min since handling + keel	27	5	268.094	280.951	3.729	0.02	6.45	0.37
age + hr since trapped + hydration	27	5	268.288	281.145	3.923	0.02	7.11	0.37

Model Structure	n	K[a]	-2 Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Evidence ratio[e]	R^2[f]
age + min since handling + hydration	27	5	268.302	281.159	3.937	0.02	7.16	0.37
age + hr since trapped + keel	27	5	268.416	281.272	4.050	0.02	7.58	0.36
age + sex + hydration	27	5	268.451	281.308	4.086	0.02	7.71	0.36
age + min since entry + keel	27	5	268.478	281.335	4.113	0.02	7.82	0.36
age + sex + keel	27	5	268.566	281.423	4.201	0.02	8.17	0.36
age + min since handling + sex	27	5	269.485	282.342	5.120	0.01	12.94	0.34
age + min since entry + min since handling	27	5	269.559	282.416	5.194	0.01	13.42	0.34
age + season + min since handling + hydration	27	6	266.244	282.444	5.222	0.01	13.61	0.41
age + hr since trapped + min since handling	27	5	269.690	282.547	5.325	0.01	14.33	0.33
intercept	27	2	280.589	285.089	7.867	-	-	-
1st Urate GCM								
age + sex	18	4	248.018	259.095	0	0.15	1.00	0.52
plasma CORT + age + sex	18	5	244.572	259.572	0.477	0.12	1.27	0.60
age + sex + season	18	5	244.769	259.769	0.674	0.11	1.40	0.60
age + min since handling + sex	18	5	245.516	260.516	1.421	0.07	2.04	0.58
plasma CORT	18	3	252.947	260.661	1.566	0.07	2.19	0.36
age + min since entry + sex	18	5	245.747	260.747	1.652	0.07	2.28	0.57
season + plasma CORT + age + sex	18	6	241.340	260.976	1.881	0.06	2.56	0.67

Model Structure	n	K[a]	-2 Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Evidence ratio[e]	R^2 [f]
plasma CORT + sex	18	4	250.054	261.131	2.036	0.06	2.77	0.46
age + season	18	4	250.554	261.631	2.536	0.04	3.55	0.44
age	18	3	254.259	261.973	2.878	0.04	4.22	0.31
plasma CORT + age	18	4	250.897	261.974	2.879	0.04	4.22	0.43
plasma CORT + season	18	4	251.235	262.312	3.217	0.03	5.00	0.42
hr since trapped + plasma CORT	18	4	251.235	262.312	3.217	0.03	5.00	0.37
plasma CORT + season + age	18	5	247.316	262.316	3.221	0.03	5.01	0.53
age + min since entry	18	4	251.621	262.698	3.603	0.03	6.06	0.41
age + hr since trapped + sex	18	5	248.013	263.013	3.918	0.02	7.09	0.52
age + min since handling	18	4	252.333	263.410	4.315	0.02	8.65	0.38
min since handling + plasma CORT	18	4	252.354	263.431	4.336	0.02	8.74	0.38
intercept	18	2	261.056	265.856	6.761	-	-	-

[a]Number of estimated parameters in the model including intercept and variance. [b]Second-order Akaike's information criterion (AIC), optimized for small sample size. [c]Difference in AICc value from that of most parsimonious model (i.e. model with lowest AICc). [d]Likelihood of the model relative to other models in the candidate set. [e]Weight of evidence that the top model is better than another model, given the candidate set. [f]Percent of variation in plasma CORT concentration (ng/mL) explained by model.

The finding of a positive influence of age on plasma CORT in wild condors in our models was somewhat surprising given that age has been previously been shown to have no effect on plasma CORT (Wilcoxen et al., 2011) or negatively correlate with feather CORT (López-Jiménez et al., 2017) in other avian species. Thus, we investigated whether age was associated with plasma CORT levels in the captive condors (n=11) not included in the above models and found that plasma CORT was not associated with age for the captive birds (Spearman's $\rho = -0.25$, $p=0.45$, SI Fig 7B). The contrasting effect of age on plasma CORT in the wild vs. captive condors may be due in part to the different sample size of the two groups (i.e., n=27 vs. 11), but also suggests that in wild condors age may be a covariate for some other influential variables that increase with age. Finkelstein et al. (Finkelstein et al., 2012) reported that condors are frequently lead poisoned over their time in the wild and we found a strong correlation between a bird's age and their time in the wild (Spearman's $\rho=0.94$, $p<.001$, n=27, SI Fig 7C) as expected for both wild fledged and released captive bred condors (23 of the wild condors were captive-bred). Thus, the influence of condor age on plasma CORT levels may be a surrogate of another influential variable present in the wild, such as lead exposure or other stressors. Finally, our finding that none of the stressor-related variables assessed here (i.e., minutes since handling start, minutes since initial pen entry, and hours since trapped in the flight pen) were significant predictors of plasma CORT levels was also somewhat surprising. However, rather than suggesting that these stressor events were not affecting condors' plasma CORT levels, we think it is more likely that plasma CORT levels were already elevated to some degree by the time of blood collection, which typically occurred ≥6 min after the start of physical

capture/handling, consistent with other avian species (Romero and Reed, 2005; Schoech et al., 1999). Thus, we conclude that our plasma collection method cannot be used to assess baseline CORT levels, or what might more accurately be defined as 'captive baseline' plasma CORT levels in California condors. Our findings also highlight the need for an alternative means to assess circulating CORT levels in condors in the absence of human-induced stress, such as urates and feathers.

3.3.2. Urate glucocorticoid metabolites

As with plasma CORT above, we investigated which factors influenced urate GCM levels, by constructing a set of possible multiple linear regression models to predict GCM concentration of the first urate sample collected after the start of handling. We chose the first urate sample as our response variable since these samples were likely the least impacted by handling stressors and were most likely to reflect a 'captive baseline' value for comparison between individuals. We included in the models the variable plasma CORT, in addition to the variables used in the models to explain plasma CORT levels above (age, sex, season, elapsed time since trapped, elapsed time since first pen entry by a technician, and elapsed time since start of handling). We did not include the body condition variables of keel and hydration status, since those variables were only of moderate to no importance in predicting plasma CORT levels, and including them further reduced the overall number of subjects available in the models. Our top models (≤1 ΔAIC units) included the parameters age, sex, plasma CORT, and season (Table 2). The majority of the 11 models with ≤3 ΔAIC units contained age or sex (nine or seven models, respectively), while five models contained plasma CORT levels, three models contained season, and one model each contained elapsed time since handling start

or time since pen entry (Table 2, SI Table 9). Only the variables age, sex, and plasma CORT levels appeared to be clearly influential for GCM levels in the first urate sample, based on the 90% confidence intervals (CI) for beta coefficients not overlapping zero (Fig 6B; SI Table 9). Consistent with this, models containing the variables age, sex and plasma CORT had combined AIC weights of 0.79, 0.66, and 0.45, respectively (SI Table 9) and thus were the most important with respect to explaining variance in the first urate GCM levels. In contrast, season and all three trapping/handling stressor variables (i.e., time since trapped, initial pen entry, or handling start) had notably lower combined AIC weights and did not significantly influence first urate GCM levels (Fig 5B; SI Table 9). Beta coefficients show that age and plasma CORT levels were positively associated with first urate GCM levels, while sex was negatively associated, with females having lower GCM levels (Fig 5B).

Age and sex were the most supported predictor variables, accounting for 52% of the variation in first urate GCM levels, while the model containing the variables age, sex, plasma CORT and season, and explains 67% (i.e., R^2 of 0.67) of the variance in first urate GCM levels (Table 2). None of the condors in our dataset were actively breeding or caring for young during sampling, so we expect that the sex and marginal seasonal differences (Fall ≥ Spring) we observed were due to inherent seasonal and sex-based variation in baseline GC or stress induced GC that have been well documented in other vertebrate species (Romero, 2002). A positive influence of plasma CORT on the first urate CGM levels indicates that circulating CORT is related to first urate GCM measurements. Since we expect that first urate GCM measurements reflect previous levels of circulating CORT, this relationship between suggests that birds that start with higher GC levels, elevate to higher GC

levels, as has been documented in great tits (*Parus major*)(Baugh et al., 2014). We interpret the fact that none of the three trapping/handling stressor variables measurably predicted first urate GCM levels as evidence that the first urate sample was not measurably impacted by these stressors. The lack of association between first urate GCM levels with handling stressors suggests that the first urate sample, collected <45 min since start of handling, but typically 25 – 73 min since initial pen entry and 19 – 141 hours since trapped is not unduly influenced by these stressor variables and thus may serve as an indicator of baseline GCM levels when compared across individuals trapped from the wild.

4. Conclusion

Collectively our findings highlight the need for careful validation when selecting an immunoassay method for hormone detection and measurement in a previously unstudied sample type or species, as well as caution when comparing immunoassay results across methods. We suggest that non-invasively collected urates and feathers hold promise for assessing condor responses to acute and chronic environmental and human-induced stressors and the MP Biomedicals ^{125}I CORT RIA kit is best for comparing hormones across sample types in the California condor.

Since endangered status of wild species can preclude the use of pharmacological challenges (i.e. ACTH) sometimes used to characterize the GC response, we suggest performing a biological method of validation for peripheral samples such as urate GCM and feather GC measurements using handling and restraint as an acute stressor. Using the RIA, we found that urate GCM and feather section CORT levels in condors generally increased following the acute stressor

event of capture and handling for biannual health checks. Despite the challenges of collecting physiological GC baseline measurements for large wild species, our results show that meaningful comparisons of GC release over time can be made between individual wild condors using peripheral samples collected during handling events. Whether this finding applies to wild-captured individuals from other free-ranging species must be similarly validated.

Future studies should aim to explain more of the variability in California condor GC measurements, and the fitness outcomes of elevation or suppression of circulating GCs in this and other critically endangered species.

5. Acknowledgements

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References

Arnold TW. Uninformative Parameters and Model Selection Using Akaike's Information Criterion. J Wildl Manage. 2010;74: 1175–1178. doi:10.2193/2009-367

Baos R, Blas J, Bortolotti GR, Marchant T a., Hiraldo F. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (*Ciconia ciconia*) exposed to heavy metal and arsenic contamination. Environ Health Perspect. 2006;114: 1497–1501. doi:10.1289/ehp.9099

Baugh AT, Oers K van, Dingemanse NJ, Hau M. Baseline and stress-induced glucocorticoid concentrations are not repeatable but covary within individual great tits (*Parus major*). Gen Comp Endocrinol. Elsevier Inc.; 2014;208: 154–163. doi:10.1016/j.ygcen.2014.08.014

Bortolotti GR, Marchant T, Blas J, Cabezas S. Tracking stress: localisation, deposition and stability of corticosterone in feathers. J Exp Biol. 2009;212: 1477–82. doi:10.1242/jeb.022152

Bortolotti GR, Marchant TA., Blas J, German T. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct Ecol. 2008;22: 494–500. doi:10.1111/j.1365-2435.2008.01387.x

Braun EJ. Osmoregulatory Systems of Birds. Sixth Edit. Sturkie's Avian Physiology: Sixth Edition. Elsevier; 2014. doi:10.1016/B978-0-12-407160-5.00012-9

Burnham KP, Anderson DR. Model Selection and Multimodel Interference: A Practical Information-Theoretic Approach. 2nd ed. New York: Springer-Verlag; 2002.

Busch DS. Measuring stress in conservation settings: A reply to Linklater. Biol Conserv. Elsevier Ltd; 2010;143: 1039–1040. doi:10.1016/j.biocon.2009.12.028

Finkelstein, Myra, Kuspa ZE, Snyder NF and Schmitt NJ. California Condor (*Gymnogyps californianus*), The Birds of North America (P. G. Rodewald, Ed.). 2015. Ithaca: Cornell Lab of Ornithology; Retrieved from the Birds of North Americ: https://birdsna.org/Species-Account/bna/species/calcon. doi: 10.2173/bna.610

Finkelstein ME, Kuspa ZE, Welch A, Eng C, Clark M, Burnett J, et al. Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis. Environ Res. 2014;134. doi:10.1016/j.envres.2014.07.022

Finkelstein ME, Doak DF, George D, Burnett J, Brandt J, Church M, et al. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc Natl Acad Sci U S A. 2012;109: 11449–54. doi:10.1073/pnas.1203141109

Finkelstein ME, George D, Scherbinski S, Gwiazda R, Johnson M, Burnett J, et al. Feather lead concentrations and (207)Pb/(206)Pb ratios reveal lead exposure history of California Condors (*Gymnogyps californianus*). Environ Sci Technol. 2010;44: 2639–47. doi:10.1021/es903176w

Gelman A. Scaling regression inputs by dividing two standard deviations. Stat Med. 2008;27: 2865–2873. doi:10.1002/sim

Harvey S, Phillips JG, Rees A, Hall TR. Stress and adrenal function. J Exp Zool. 1984;232: 633–45. doi:10.1002/jez.1402320332

Herring G, Ackerman JT, Herzog MP. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ Sci Technol. 2012;46: 6339–6346. doi:10.1021/es300668c

Herring G, Gawlik DE. Stability of Avian Fecal Corticosterone Metabolite Levels in Frozen Avian Feces. J Wildl Manage. 2009;73: 1010–1013. doi:10.2193/2008-398

Hirschenhauser K, Spreitzer K, Lepschy M, Kotrschal K, Möstl E. Excreted corticosterone metabolites differ between two galliform species, Japanese Quail and Chicken, between sexes and between urine and faecal parts of droppings. J Ornithol. 2012;153: 1179–1188. doi:10.1007/s10336-012-0848-9

Jachowski DS, Washburn BE, Millspaugh JJ. Revisiting the importance of accounting for seasonal and diel rhythms in fecal stress hormone studies. Wildl Soc Bull. 2015;39: 738–745. doi:10.1002/wsb.592

Jankowski MD, Wittwer DJ, Heisey DM, Franson JC, Hofmeister EK. The adrenocortical response of greater sage grouse (*Centrocercus urophasianus*) to capture, ACTH Injection, and confinement, as measured in fecal samples. Physiol Biochem Zool. 2009;82: 190–201. doi:10.1086/596513.The

Keay JM, Singh J, Gaunt MC, Kaur T. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J Zoo Wildl Med. 2006;37: 234–44. doi:10.1638/05-050.1

Khan MZ, Altmann J, Isani SS, Yu J. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen Comp Endocrinol. 2002;128: 57–64.

Koren L, Nakagawa S, Burke T, Soma KK, Wynne-edwards KE, Geffen E. Non-breeding feather concentrations of testosterone, corticosterone and cortisol are associated with subsequent survival in wild house sparrows. Proc R Soc Biol Sci. 2012;279: 1560–1566. doi:10.1098/rspb.2011.2062

Kouwenberg A, McKay DW, Fitzsimmons MG, Storey AE. Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: feather corticosterone levels of food-supplemented Atlantic Puffin chicks. J F Ornithol. 2015;86: 73–83. doi:10.1111/jofo.12090

Landys MM, Ramenofsky M, Wingfield JC. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen Comp Endocrinol. 2006;148: 132–149. doi:10.1016/j.ygcen.2006.02.013

Legagneux P, Gauthier G, Chastel O, Picard G, Bêty J. Do glucocorticoids in droppings reflect baseline level in birds captured in the wild? A case study in snow geese. Gen Comp Endocrinol. Elsevier Inc.; 2011;172: 440–445. doi:10.1016/j.ygcen.2011.04.009

López-Jiménez L, Blas J, Tanferna A, Cabezas S, Marchant T, Hiraldo F, et al. Lifetime variation in feather corticosterone levels in a long-lived raptor. Oecologia. 2017;183. doi:10.1007/s00442-016-3708-0

Madliger CL, Semeniuk CAD, Harris CM, Love OP. Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker. Biol Conserv. 2015;192: 409–417. doi:10.1016/j.biocon.2015.10.021

McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Horm Behav. 2003;43: 2–15. doi:10.1016/S0018-506X(02)00024-7

Miller R, Plessow F, Rauh M, Gröschl M, Kirschbaum C. Comparison of salivary cortisol as measured by different immunoassays and tandem mass spectrometry. Psychoneuroendocrinology. 2013;38: 50–57. doi:10.1016/j.psyneuen.2012.04.019

Millspaugh JJ, Washburn BE. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen Comp Endocrinol. 2004;138: 189–99. doi:10.1016/j.ygcen.2004.07.002

Möstl E, Rettenbacher S, Palme R. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Ann N Y Acad Sci. 2005;1046: 17–34. doi:10.1196/annals.1343.004

Nakagawa S, Möstl E, Waas J. Validation of an enzyme immunoassay to measure faecal glucocorticoid metabolites from Adelie penguins (*Pygoscelis adeliae*): a non-invasive tool for estimating. Polar Biol. 2003;26: 491–493. doi:10.1007/s00300-003-0506-z

Nilsson PB, Hollmén TE, Atkinson S, Mashburn KL, Tuomi PA, Esler D, et al. Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (*Histrionicus histrionicus*). Comp Biochem Physiol - A Mol Integr Physiol. 2008;149: 275–283. doi:10.1016/j.cbpa.2008.01.002

Palme R, Rettenbacher S, Touma C, El-Bahr SM, Möstl E. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann N Y Acad Sci. 2005;1040: 162–71. doi:10.1196/annals.1327.021

Raff H, Homar PJ, Burns EA. Comparison of two methods for measuring salivary cortisol. Clin Chem. 2002;48: 207–208.

Rettenbacher S, Möstl E, Hackl R, Ghareeb K, Palme R. Measurement of corticosterone metabolites in chicken droppings. Br Poult Sci. 2004;45: 704–11. doi:10.1080/00071660400006156

Romero LM, Fairhurst GD. Measuring corticosterone in feathers: Strengths, limitations, and suggestions for the future. Comp Biochem Physiol -Part A Mol Integr Physiol. Elsevier Inc.; 2016;202: 112–122. doi:10.1016/j.cbpa.2016.05.002

Romero LM, Reed JM. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? Comp Biochem Physiol - A Mol Integr Physiol. 2005;140: 73–79. doi:10.1016/j.cbpb.2004.11.004

Romero LM. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen Comp Endocrinol. 2002;128: 1–24.

Schoech SJ, Romero LM, Moore IT, Bonier F. Constraints, concerns and considerations about the necessity of estimating free glucocorticoid concentrations for field endocrine studies. Funct Ecol. 2013;27: 1100–1106. doi:10.1111/1365-2435.12142

Schoech SJ, Ketterson ED, Nolan V, Jr VN. Exogenous testosterone and the adrenocortical response in dark-eyed juncos. Auk. 1999;116: 64–72. doi:10.2307/4089454

Sheriff MJ, Dantzer B, Delehanty B, Palme R, Boonstra R. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia. 2011;166: 869–87. doi:10.1007/s00442-011-1943-y

Staley AM, Blanco JM, Dufty AM, Wildt DE, Monfort SL. Fecal steroid monitoring for assessing gonadal and adrenal activity in the golden eagle and peregrine falcon. J Comp Physiol B. 2007;177: 609–22. doi:10.1007/s00360-007-0159-2

Tempel D, Gutiérrez R. Factors related to fecal corticosterone levels in California spotted owls: implications for assessing chronic stress. Conserv Biol. 2004;18: 538–547. doi:10.1111/j.1523-1739.2004.00372.x

Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann N Y Acad Sci. 2005;1046: 54–74. doi:10.1196/annals.1343.006

United States Fish and Wildlife Service. California Condor 5-Year Review : Summary and Evaluation. 2013.

Virgolini MB, Chen K, Weston DD, Bauter MR, Cory-Slechta D a. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol Sci. 2005;87: 469–82. doi:10.1093/toxsci/kfi269

Walker BG, Boersma PD, Wingfield JC. Field endocrinology and conservation biology. Integr Comp Biol. 2005;45: 12–8. doi:10.1093/icb/45.1.12

Warnken T, Huber K, Feige K. Comparison of three different methods for the quantification of equine insulin. BMC Vet Res. BMC Veterinary Research; 2016;12: 196. doi:10.1186/s12917-016-0828-z

Washburn BE, Morris DL, Millspaugh JJ, Faaborg J, Schulz JH. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. Condor. 2002;104: 558–563.

Wasser SK, Hunt KE, Brown JL, Cooper K, Crockett CM, Bechert U, et al. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen Comp Endocrinol. 2000;120: 260–275. doi:10.1006/gcen.2000.7557

Wilcoxen TE, Boughton RK, Bridge ES, Rensel M a, Schoech SJ. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. Gen Comp Endocrinol. Elsevier Inc.; 2011;173: 461–6. doi:10.1016/j.ygcen.2011.07.007

Wingfield JC, Kitaysky AS. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr Comp Biol. 2002;42: 600–9. doi:10.1093/icb/42.3.600

Wingfield JC, Ramenofsky M, Pottinger TG. Stress Physiology in Animals. 1st ed. Balm PHM, editor. Boca Raton: CRC Press LLC; 1999.

CHAPTER 4: THE INFLUENCE OF LONG-TERM CONTAMINANT EXPOSURE ON THE STRESS RESPONSE OF THE CALIFORNIA CONDOR

Abstract

The vertebrate glucocorticoid stress response is a critical physiological adaptation for maintaining energy homeostasis and is known to be disrupted by lead exposure. The California condor, a closely monitored and frequently lead-exposed species, provides a unique opportunity to investigate the effects of lead on the avian glucocorticoid response. We used radioimmunoassay to measure plasma corticosterone and urate glucocorticoid metabolites (GCM) in samples collected at ~15 minute intervals after a handling and blood collection stressor during routine health checks for captive and wild California condors. We assessed GCM concentrations of the first urate sample collected, peak urate sample collected within 2 hours of handling start, and the magnitude differences between these values (Δurate GCM). Wild condors exhibited greater peak urate GCM levels (2250 ± 1440 ng/g dry wt, average +/- SD, n=27) than captive condors (907 ± 489 ng/g dry wt.,U = 28, p = 0.003). No significant differences between wild and captive individuals were observed for plasma corticosterone levels (U=153, p=0.11, Fig 1A) or first urate GCM levels (U=101, p=0.62).We then assembled a suite of multiple linear regression models and used an information theoretic approach (AIC_c) to identify other important variables (e.g., age, sex, time since the handling stressor, lead exposure; Table 1) associated with variation in plasma corticosterone and urate GCM levels in wild condors. Results show that lead exposure variables based on blood lead data were not predictive of plasma corticosterone or urate GCM levels. Days absent from management area, a behavior associated with higher lead exposure risk and reduced survival, had a positive effect

on plasma corticosterone levels (β = 53 ± 20 SE) and peak urate GCM levels (β = 1090 ± 586 SE). Duration of pre-sampling captivity, time between first technician entry into the flight pen and handling start, total years observed feeding on marine mammals were also predictive of stress-induced glucocorticoid levels. Differences in pre-sampling captivity and technician entry explain more variance in condor GC response outcomes (~25%) than differences in contaminant risk (~15%). Our results indicate that exposure to lead and persistent organic pollutants may be altering condor stress physiology. This work addresses a critical lack in our understanding of how long-term contaminant exposure impacts the California condor recovery effort, and the population health of other scavenging species exposed to lead-based ammunition worldwide.

1. Introduction

The California condor (*Gymnogyps californianus*), one of the world's most critically endangered species, is frequently lead poisoned via ingestion of spent lead-based ammunition (Church et al. 2006, Cade 2007, Finkelstein et al., 2012). In fact, lead toxicosis is the leading cause of mortality in adult California condors (Rideout et al., 2012). While lead-related deaths can be quantified, the sub-lethal effects of chronic lead exposure are more difficult to assess in wildlife (Hunt, 2012). The current management of the California condor provides an important and unique opportunity for capturing sub-lethal effects in a free-ranging species because intensive exposure monitoring provides a wealth of data on each individual's clinical history, daily movements, and feeding behaviors.

Lead exposure causes dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, the part of the endocrine system responsible for modulating the release of glucocorticoid (GC) hormones in response to stress (e.g., Virgolini et al. 2006, Gump et al. 2008, Rossi-George et al. 2009, Fortin et al. 2012). In avian species, glucocorticoids induce metabolic and behavioral changes important for meeting energy demands and avoiding dangerous situations (Wingfield and Kitaysky, 2002), but when these hormones are present in inappropriate concentrations, decreased survival and fitness have been documented (Ellenberg et al., 2007; Owen et al., 2012; Schoenle et al., 2017).

Condors are trapped up one or two times per year for routine health checks. Health checks are not standardized for GC sample collection, and condors experience varying durations of time in flight pens after trap-up before the health check. Additionally, the need to net and physically restrain these large birds to obtain a blood sample makes it inherently challenging to measure pre-stress GC levels, as circulating GC levels in birds are known to elevate within minutes after a stressor (Newman et al., 2017; Romero and Reed, 2005). GC metabolite concentrations (GCM) in excreta have been established as suitable peripheral samples for measurement of circulating glucocorticoids in wildlife (Sheriff et al., 2010). The advantage of using the peripheral samples is the lag-time between elevation in circulating GC and elevation in GCM levels in excreta due to metabolism and gut passage time (Möstl et al., 2005; Rettenbacher et al., 2004), and thus pre-stress samples may more accurately reflect pre-stress GC levels. Foundational work presented in the third chapter of my dissertation established that measurable

elevation in GC after a capture and handling event can be detected in sequentially collected California condor urates.

In light of the epidemic rates of lead exposure and lead poisoning in condors, and the potential for lead exposure to impact the stress response in condors, we conducted a field study to investigate the influence of known and predictive measures of lead exposure, along with other life history and behavioral variables on GC levels measured in plasma and urate samples collected from wild and captive condors during biannual California condor health checks. To our knowledge, only two studies have found associations between lead exposure and GC levels in an avian model (Baos et al., 2006; Meillère et al., 2016) and ours is the first investigation to utilize extensive daily monitoring records to explore the effect of lead on the stress response, both plasma corticosterone (CORT) and urate GCM levels in serially collected samples, in a long-lived avian scavenger. Years a condor was observed marine mammal feeding is strongly associated (Adj. R^2=0.81, F=92.05, df=20, p<0.0001*)* with plasma concentrations of persistent organic pollutants (POPs, including polychlorinated biphenyls (PCBs)) in condors (Kurle et al., 2016) and as PCBs are known to alter the avian stress response (S. Tartu et al., 2015a) this variable was also included in our analyses.

Our work provides insight into the potential for lead-induced physiological effects in avian scavengers worldwide known to be exposed to lead via food sources contaminated with spent-lead-based ammunition (Bellinger et al., 2013; Johnson et al., 2013; Pain, 2009). We also highlight the inherent difficulty in comparing GC responses from wild-caught condors that undergo multiple stressors in the capture

process before sample collections. We suggest ways to control for this variation and avoid pitfalls in interpretation of GC data.

2. Materials and Methods

2.1 Study subjects

Wild condors (n=41) from central California, managed by Pinnacles National Park and Ventana Wildlife Society, and captive condors housed at the Los Angeles Zoo and Botanical Gardens (n = 7) and Santa Barbara Zoo (n = 4) were sampled for plasma and urates between 2013 and 2016. Both male and female condors were included, with ages ranging 1-36 years (Table S1). The use of vertebrate samples for this research was approved by The University of California Santa Cruz's Institutional Animal Care and Use Committee with permission from Los Angeles Zoo and Botanical Gardens and Santa Barbara Zoo (IACUC office approval code FINKM1307). Samples were collected at Pinnacles National Park and Ventana Wildlife Society under USFWS sub-permits (Pinnacles National Park Permit # TE 157291-1; Ventana Wildlife Society Permit # TE-026659-14).

2.2 Stress challenge and sample collections

Plasma and urates were collected during scheduled handling events for routine health monitoring as part of condor management protocols (United States Fish and Wildlife Service, 2013). Wild condors were passively captured using carcass-baited double door traps, operated by technicians concealed within a blind to prevent detection of human presence at the trap. Birds were then given access through another blind-operated door to a larger, netted, flight pen (dimensions ~7.5 m x 12 m x 6 m tall, Table S2 and S3). Birds were typically held in the larger flight

pen for >24 hr (range=19-223 hr, median=45 hr, Table S2 and S3) before handling commenced. Captive birds were housed permanently in flight pens of similar dimensions and design. Condors in flight pens were normally housed in groups of 2-9 individuals and had access to food and water *ad libitum*, or in the case of captive individuals, access to food on a weekly basis with set fast days to mimic condor feeding behavior in the wild. While in the flight pen, condors were visually isolated from humans until the day of handling.

On the day of handling, the time of initial pen entry by technicians was recorded for each handling event. Following pen entry by technicians, condors were herded either onto the ground or into enclosed isolation pens, a process that can take 2-15 minutes. Condors were captured sequentially and individuals held later in the day often experienced multiple pen entries by technicians. Handling start was recorded upon a bird's capture by a large, hand-held hoop net and time since first technician entry was recorded (median=61 min, range=10-200 min, Table S2 and S3). The condor was then restrained in hand for a median time of 27 min (range=14-47 min, Table S3) for blood draw, feather collection, physical examinations, and in the case of wild birds, tracking equipment maintenance.

2.2.1 Plasma collection

Blood samples were collected within 3-18 min of handling start (median= 6 min). Blood (1 – 2 mL) was collected from the metatarsal vein into heparinized BD Vacutainers® and placed on ice. Plasma was separated from whole blood via centrifugation (10 min at 2,000 x g) within 12 hr of blood collection, transferred into cryovials, and stored at -80˚C until analysis.

2.2.2. Kenneling and urate collection

Immediately after handling as described above, condors were placed into a modified dog kennel with a raised vinyl-coated mesh floor above a removable Plexiglas tray to collect urate samples. As with other new world vulture species, California condors perform urohidrosis, and generally excrete fecal (i.e. solid) and urate (i.e. liquid) material separately (Finkelstein et al., 2015). Since urates were collected more frequently during condor kenneling time periods, we chose to use GCM levels in urates for comparison across individuals in this study.

The environment inside the kennel was low light and visually isolated from outside stimuli. The kennel had sufficient room to allow the condor to turn around and lay in repose. Wild birds were held within kennels for as long as logistically feasible (at least 2 hrs), before release to the wild or transport to clinics for treatment of lead poisoning or other medical conditions if warranted. Condors did not have access to food and water while in the kennel. Urate samples were serially collected from the sub-floor tray which was checked at 15 min intervals while birds were kenneled. However, if urate excretion was detected (by sound) before the 15 min tray check, it was collected immediately. Urates were placed on dry ice within 30 min of defecation and stored at -80˚C until extraction to arrest hormone metabolism by bacterial enzymes (Glucs et al. 2018, in review, SI Fig 2, Möstl et al., 2005; Touma and Palme, 2005).

2.3. Sample processing and hormone extraction

2.3.1. Plasma processing

Plasma samples were diluted in radioimmunoassay kit buffer using half volumes of all samples as described and validated for avian plasma in Washburn et al. (2002) and for condor plasma in Glucs et al. (2018, in review).

2.3.2. Urates processing and extraction

As described in Chapter 3, whole urate samples were lyophilized and re-suspended in 80% methanol (Certified ACS, A412-4, Fisher Scientific) at a ratio of 75 mL:1 g dry weight (adapted from methods described by Herring and Gawlik, 2009; Rettenbacher et al., 2004; Tempel and Gutiérrez, 2004). The sample-solvent mixture was vortexed for 15 sec, shaken at room temperature for 30 min on an orbital table top shaker, and then centrifuged at 2,500 x g for 15 min. Methanol supernatant was transferred to a new vial, evaporated to dryness under vacuum or nitrogen at room temperature, and stored at -80˚C until analysis. Extraction efficiency of a CORT standard (Enzo Life Sciences, NY) was 98%.

2.4. Corticosterone and glucocorticoid metabolite measurement methods

We employed a CORT I^{125} double antibody radioimmunoassay kit (Prod. No. 07120103, MP Biomedicals) to measure plasma CORT and urate GCM concentrations (validated in Chapter 3). The manufacturers' protocols were followed, with samples being re-suspended and diluted and analyzed in accordance to the manufacturer's instructions, with the exception of using half volumes of all kit reagents as described in Washburn et al. (2002).

An 'analytical consistency' sample was generated by pooling aliquots of condor plasma samples together, thoroughly mixing, sub-aliquoting into vials and storing at -80°C; an aliquot of this consistency plasma sample was run in triplicate in each assay to assess intra-assay variability (%RSD = 3.2) and between assay

reproducibility (%RSD = 4.1). Nine urate samples were run in two different analytical batches with separate standard curves to provide a percent difference measurement (% diff) for inter-assay reproducibility (Avg % diff = 6.8). Plasma and urate samples were also serially diluted and run to test for parallelism with the standard curve (Chapter 3 SI Fig 1). Standard curves for each assay day were generated by fitting measurements from serially diluted CORT standards to a 4-parameter logistic curve; CORT and GCM concentrations in samples were calculated from these standard curves. Reported non-target compound cross-reactivities reported for the radioimmunoassay kit antibody were < 1% for all steroids tested, with deoxycorticosterone having the highest tendency for cross reaction at 0.34% (kit manual, MP Biomedical).

2.5 Lead exposure and behavior data

Data on lead exposure, feeding, and movement behavior were collected by Pinnacles National Park and Ventana Wildlife Society and used to define variables in Table 1. Condor trapping and blood testing takes place at these release sites two times per year (roughly during Spring and Fall seasons), and most individuals are captured and tested at least once per year. To account for the differences in exposure monitoring for each individual, we calculated the proportion of clinical blood lead tests (>35 µg/dL) observed over the previous 18 months (PropHiPb18) and over lifetime (PropHiPbLife) (Table 1). Years a condor was observed marine mammal was also included in our analyses, to account for the potential of persistent organic pollutants to alter glucocorticoid release in condors.

Table 1. Variables considered in predictive models for GC response outcomes in California condors

Variable	Description	Rationale
All condors		
Status	Binary variable indicating wild or captive. Wild: free flying in Pinnacles National Park or Ventana Wildlife Society populations, Captive: in zoo population	Controls for differences in GC response between free-flying vs. zoo-captive California condors. Additionally, captive condors are not exposed to the environmental contaminants present in the diet of wild condors.
Age	Condor age in years	Controls for potential changes in GC release with condor age (e.g. Wilcoxen et al., 2011)
Sex	Binary variable indicating sex: Male or female	Controls for potential gender differences in GC release (e.g. Hirschenhauser et al., 2012)
Season	Binary variable for season of sample collection. Wet season: Nov1-May 31, Dry season: Jun1-October 31	Controls for potential seasonal differences in GC release due to weather conditions or potential life history stages (Romero, 2002)
FlightPen	Hours spent in flight pen before first technician entry to flight pen on the day of handling (wild condors only)	Accounts for effect of different time periods captive in flight pen before handling commences (Dickens et al., 2009)
TechEntry	Minutes elapsed between first technician entry to flight pen on the day of handling and handling start (hand-netting) for individual condor	Accounts for variation in time elapsed between first pen entry by technician and the start of handling. Witnessing human interactions with conspecifics can increase GC levels (e.g. Jones et al., 2016)
MinSinceHandl	Minutes elapsed between handling start and sample collection (plasma and first urate only)	Accounts for effect of differences in time elapsed between handling start and sample collection for plasma and first urate samples. Also encapsulates handling duration for first urate GCM models as sampling begins after handling bout ends (e.g. Newman et al., 2017).
RestraintDur	Restraint duration for health check in minutes from handling start to kenneling (peak and ∆urate only)	Accounts for effect of restraint duration on maximum elevation of urate GCM collected within 2 hrs of handling start

Variable	Description	Rationale
Wild condors only		
CurrentPb	Blood lead level (µg/dL) at time of handling event	Tests whether current lead level effects GC response to handling (Baos et al., 2006).
PropHiPbLife	Proportion of blood tests > clinical lead level (35 µg/dL) over lifetime	Approximates cumulative lead poisonings and clinical treatments over lifetime
PropHiPb18	Proportion of blood tests > clinical lead level (35 µg/dL) over 18 months before handling event	Approximates cumulative recent lead poisonings and clinical treatments over past 18 months
YrsMMFed	Years observed feeding on marine mammals over lifetime	Approximates cumulative exposure to endocrine-disrupting POPs (DDE, PCBs, PBDE). Strong predictor of plasma levels for these contaminants in California condors (Kurle et al., 2016). Lifetime exposure most relevant since these contaminants are bioaccumulative.
DaysAbsent	Free-flying days absent from monitoring (not visually sighted or observed via radio-telemetry) over lifetime	Approximates lead exposure risk over lifetime. Birds foraging outside the management area may be more likely to encounter lead-contaminated carcasses. Days absent over previous 2 months associated with decreased survival probability in California condors (Bakker et al., 2016).
DaysAbsent18	Free-flying days absent from monitoring (not visually sighted or observed via radio-telemetry) over 18 months before handling event	Approximates recent lead exposure risk over previous 18 months. See above.

2.6. Statistical Analysis and Model Selection

Data analyses were performed using R 3.4.3 (R Core Development Team, 2011). The plasma CORT response outcome is reported as nanograms per milliliter (ng/mL). Urate series from each handling event were summarized by three response outcomes reported as nanograms per gram dry weight (ng/g dry wt). 'First urate GCM' is the GCM concentration of the first sample collected in series used to determine GCM levels at start of sample collection, a potential baseline GC measurement. 'Peak urate GCM' is the maximum sample GCM concentration collected within 2 hours of handling start and from handling events where samples were provided 1-2 hours since handling start (represents maximal GC release). 'Delta urate GCM' is the difference in GCM concentration between 'peak urate GCM' and 'first urate GCM' providing a measurement of maximal change in GC levels over time for each individual. We use Spearman's ρ correlations to determine relationships between plasma CORT levels and urate GCM levels as well as to identify covariance between predictor variables. Mann-Whitney U/Wilcoxon tests were employed for assessing GC differences between wild and captive condors. A significance level of <0.05 was used for null hypothesis rejection.

To determine which predictor variables influenced condor plasma CORT and urate GCM levels we used a stepwise information theoretic approach (Burnham and Anderson, 2002) on four sets of multiple regression models. Values of continuous predictor variables were scaled before regressions by dividing by two times the standard deviation, so the magnitude of coefficients could be directly compared

(Gelman, 2008). We then constructed and ranked *a priori* models for predicting plasma CORT and urate GCM levels using second order Akaike's Information Criterion (AICc) to control for small sample sizes (R package: AICcmodavg; Mazerolle, 2017). In the first round of selection the candidate model set included the variables age, sex, season, FlightPen, TechEntry, MinSinceHandl, RestraintDur (Table 1). Status (captive or wild) was included as a predictor variable when captive and wild condors were included in a dataset. Model sets included global models with all variables, and an intercept-only model. The models with the lowest AIC_c value were considered the most parsimonious (Burnham and Anderson, 2002). Variables in these top models (within top 90% of AIC_c weights and AIC_c at least 1 unit < null model AIC_c) were included as potential additive effects in the second set of models for determining influence of lead exposure variables and behavioral predictors of exposure risk on condor GC levels (Tables S5 and S6). Age was retained for plasma CORT models (AIC_c wt =0.41), MinSinceHandl for first urate GCM models (AIC_c wt. = 0.29), TechEntry (AIC_c wt.=0.61) and FlightPen (AIC_c wt. = 0.43) for peak urate GCM, and TechEntry to (AIC_c wt. = 0.80) and FlightPen (AIC_c wt. = 0.69) for Δ urate GCM (Tables S5 and S6).

In our subsequent model set for investigation of behavioral and contaminant exposure predictors on condor GC levels, we initially retained the variables from the top predictor model from the previous round of model selection for each response outcome, but also considered reduced forms without these variables (Table S7).

For all models we calculated differences in AIC_c from the top model (ΔAIC_c) and AIC_c weights. The subset of models that made up 90% of AIC_c weights of the complete model set and were >1 AIC_c better than the null model were included in our

model averaging to infer the influence of each variable (R package: AICcmodav; Mazerolle, 2017; Table 2). For these final candidate model sets we summed the AIC_c weights for each variable of influence and calculated their model averaged beta coefficients to estimate the direction and strength of their effect on GC levels (Lukacs et al., 2010; Table S8). While 85% confidence intervals (CIs) are recommended for excluding uninformative variables in AIC_c selected models with large sample sizes ($n/K > 40$) (Arnold, 2010), our smaller sample sizes ($n/K < 15$) necessitated larger CIs for conservative assessment of our model averaged beta coefficient estimates. The reported 90% CIs tended to retain informative variables form the best approximating models, and exclude variables with low sum AIC_c weights ($w < 0.20$). Model fit was assessed by calculating R^2 values and examining residuals of the global model for influential variables.

Table 2. Top models from 2nd model selection step with lead exposure and behavior variables included for prediction of wild condor GC response outcomes. Selection criteria for candidate models was to be within summed AIC_c wt of 0.90 and >1 ΔAIC_c from intercept-only model.

Model Structure	n	K[a]	Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Evidence ratio[e]	R^2[f]
Plasma CORT								
DaysAbsent	41	3	-203.374	413.397	0.000	0.48	1.00	0.28
DaysAbsent + Age	41	4	-202.557	414.225	0.828	0.32	1.51	0.33
global model	41	5	-202.169	416.053	2.656	0.13	3.77	0.21
YrsMMFed	41	3	-205.311	417.270	3.873	0.07	6.94	0.22
intercept	41	2	-210.245	424.806	11.410	0.00	300.32	--
First urate GCM								
min since handling start	33	3	-250.518	507.8634	0.00	0.68	1.00	0.11
intercept	33	2	-252.497	509.3947	1.53	0.32	2.15	--
Peak urate GCM								
FlightPen + TechEntry + DaysAbsent	27	5	-226.848	466.553	0.000	0.408	1.00	0.42
FlightPen + TechEntry + YrsMMFed	27	5	-227.005	466.866	0.313	0.349	1.17	0.41
FlightPen + TechEntry + DaysAbsent18	27	5	-228.237	469.331	2.777	0.102	4.01	0.36
FlightPen + TechEntry	27	4	-230.107	470.031	3.478	0.072	5.69	0.26
YrsMMFed	27	3	-232.274	471.591	5.037	0.033	12.41	0.13
global model	27	7	-226.403	472.701	6.148	0.019	21.63	0.44
intercept model	27	2	-234.139	472.7773	7.96098	0.01	53.54	--

Model Structure	n	K[a]	Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Evidence ratio[e]	R^2 [f]
Δ urate GCM								
FlightPen + TechEntry + YrsMMFed	27	5	-225.98	464.8164	0	0.40	1.00	0.45
FlightPen + TechEntry + DaysAbsent	27	5	-226.109	465.0748	0.258471	0.35	1.14	0.45
FlightPen + TechEntry + DaysAbsent18	27	5	-227.116	467.0899	2.273572	0.13	3.12	0.41
FlightPen + TechEntry	27	4	-228.924	467.6656	2.849224	0.10	4.16	0.32
global model	27	7	-225.331	470.5571	5.740759	0.02	17.64	0.48
intercept model	27	2	-234.139	472.7773	7.96098	0.01	53.54	--

[a]Number of estimated parameters in the model including intercept and variance. [b]Second-order Akaike's information criterion (AIC), optimized for small sample size. [c]Difference in AIC_c value from that of most parsimonious model (i.e. model with lowest AIC_c). [d]Likelihood of the model relative to other models in the candidate set. [e]Weight of evidence that the top model is better than another model, given the candidate set. [f]Percent of variation in GC concentration explained by model.

3. Results

3.1. Glucocorticoid Measurements

California condor plasma CORT concentrations ranged from 1.0 – 190 ng/mL (median=73 ng/mL, n=52 samples, 41 condors, Table S2), and urate GCM concentrations ranged from 0.74 – 7200 ng/g dry wt. (median= 810 ng/g dry wt., n=333 samples, 35 condors Table S3). Wild condors exhibited greater peak urate GCM levels (median= 1900 ng/g dry wt., n=27 samples) than captive condors (median=1100 ng/g dry wt., n=7 samples) (U = 28, p=0.003, Fig 1C). Similarly the Δ urate GCM levels over 2 hours was greater in wild condors (median=990 ng/g dry wt., n=27 samples) than in captive condors (median=274.9, n=7 samples)(U = 23, p=0.002, Fig 1D). No significant differences between wild and captive individuals were observed for plasma CORT levels (U=153, p=0.11, Fig 1A) or first urate GCM levels (U=101, p=0.62, Fig 1B).

3.2. Variables influencing glucocorticoid response outcomes

For all GC response outcomes, the variables Sex, Season, RestraintDur, CurrentPb, PropHiPbLife, PropHiPb18 were not present in models within the top 0.90 of summed AIC_c weights (Table S7), indicating that these variables are not predictive of GC response outcomes in our California condor dataset.

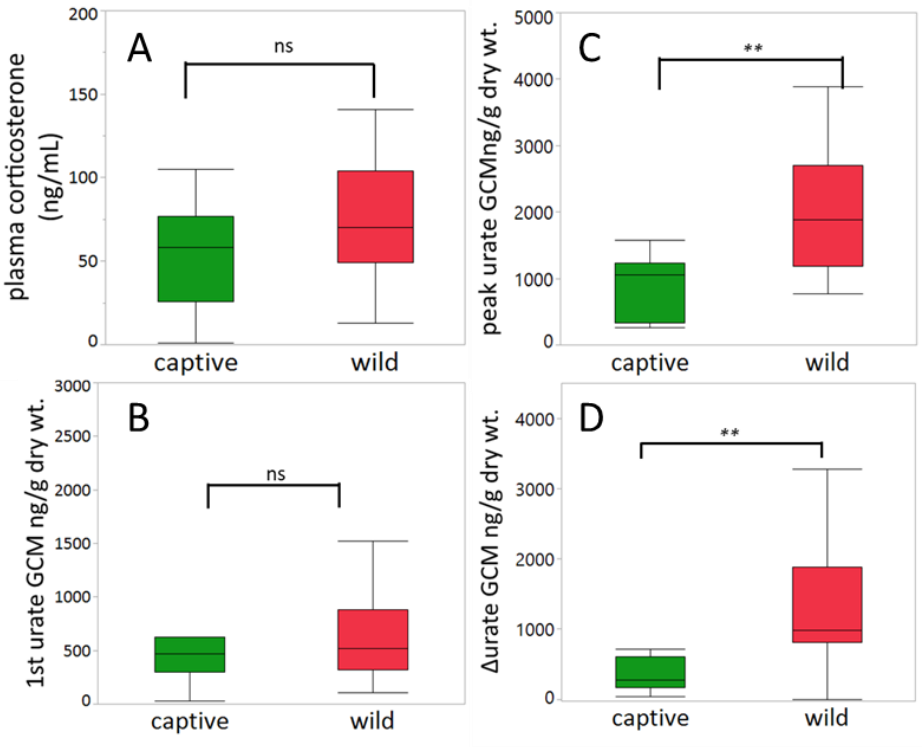


Figure 1. Wild condor peak and Δ urate GCM levels more elevated than in captive condors. (A) No significant differences between wild and captive individuals were observed for plasma CORT levels (U=153, p=0.11,) (B) or first urate GCM level (U=101, p=0.62). (C) Wild condors exhibited greater peak urate GCM level (median= 1900 ng/g dry wt., n=27) than captive condors (median=1100 ng/g dry wt., n=7) (U = 28, p=0.003, Fig 1C). (D) Δ urate GCM level over 2 hours was greater in wild condors (median=990 ng/g dry wt., n=27) than in captive condors (median=274.9, n=7)(U = 23, p=0.002). Labels 'ns' indicate no significant difference (p>0.05) and '**' indicate p<0.01. First urate GCM levels were positively correlated with plasma CORT levels collected during the same handling event (r_S=0.50, p=0.007). Peak urate GCM levels and Δ urate GCM levels however where not significantly correlated with plasma CORT levels (p=0.062 and p=0.55 respectively). Notably, first urate GCM levels were positively correlated with peak urate GCM levels collected from the same individual (r_S=0.49, p=0.003), but not with Δurate GCM levels (r_S=0.036, p=0.84). This latter result shows that birds with higher first urate GCM levels reached higher peak GCM levels within 2 hours, but absolute change in GC level was unrelated to the starting GC levels in these individuals.

3.2.1. Variables influencing condor plasma CORT levels

The most parsimonious model for plasma CORT levels contained the DaysAbsent variable, which is a behavior associated with increased probability of lead poisoning in this condor population (Bakker, unpub.), with an AIC_c weight of 0.48. This model, however, explained only 28% of variation in plasma CORT levels, indicating substantial unexplained variation. The second-best model containing Age and DaysAbsent also had reasonable support (<1ΔAIC_c) (Table 2). The weight of evidence indicates that these models were >3 times more likely than the remaining candidate models that included YrsMMFed (Table 2). After model averaging beta coefficient estimates and standard errors for all candidate models, the intercept-only model, and the global model containing all top variables, only DaysAbsent remained significant (β = 53 ± 20 SE, 90% CI: 20.9-85.4, Fig 1A, Table S8).

3.2.1. Variables influencing urate GCM levels

Only MinSinceHandl had support as a predictor of first urate GCM level (β = 348 ± 175 SE, 90% CI: 115-144, Fig 1B, Table S8), suggesting that this variable includes an influence of short-term stressors rather than a pure measurement of baseline GCM level. However, this model only explained 11% of the variance in first urate GCM level (Table S6). There was no support for the other variables as predictors of first urate, including measures of lead exposure.

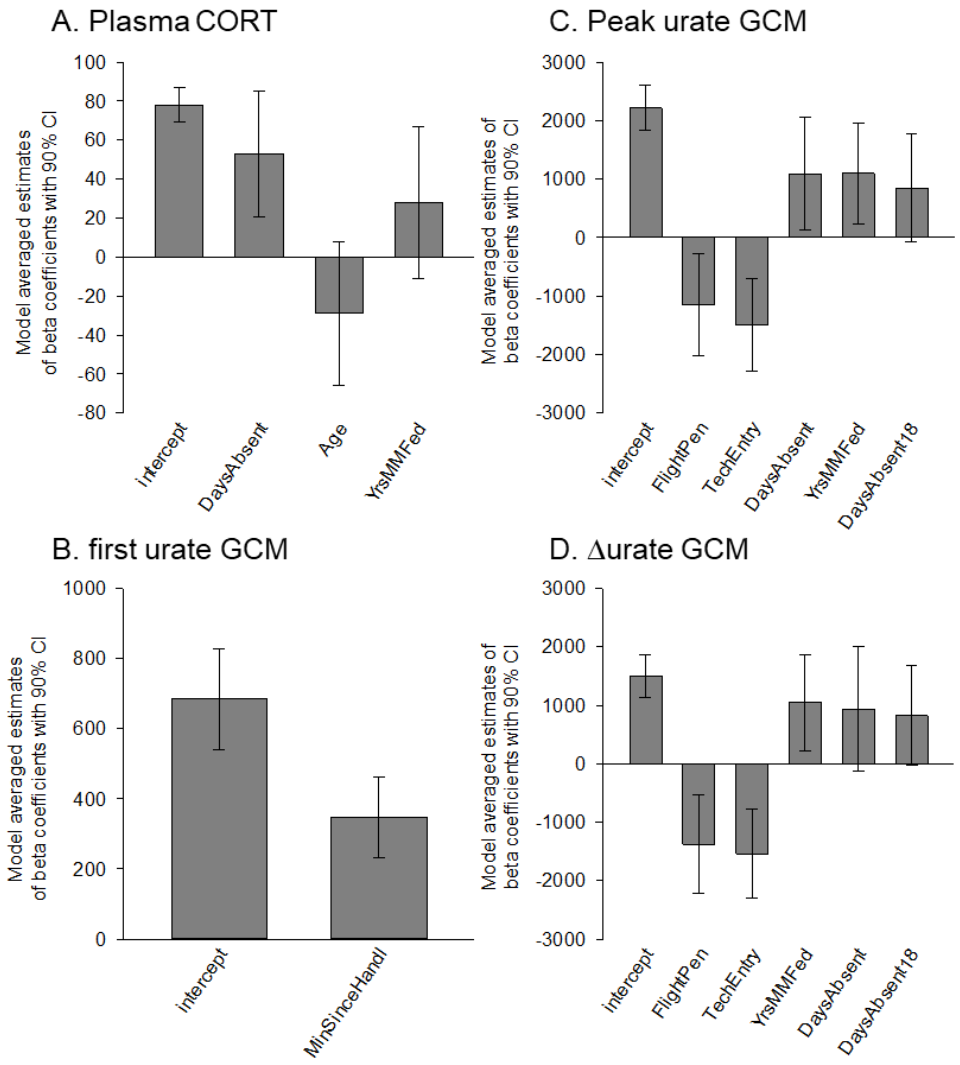


Figure 2. Model averaged beta estimates for variables present in the top AIC models for each glucocorticoid response outcome: (A) Plasma corticosterone (CORT) levels, (B) first urate glucocorticoid metabolites (GCM), (C) Peak urate GCM, (D) Δurate GCM. Error bars represent 90% CI based on model-averaged standard error. CI including zero indicate significant model uncertainty for direction of effect. See Table 1 for variable definitions and Table S8 for model averaged beta coefficients, standard error and CIs.

Of the candidate models explaining variation in peak urate GCM levels, the most parsimonious model consisted of two stressor variables (FlightPen and TechEntry) and DaysAbsent, had an AIC_c weight of 0.41, and explained 42% of GCM variance (Table 2). The second-best model containing FlightPen, TechEntry, and YrsMMFed also had reasonable support (<1ΔAIC_c, Table 2). By weight of evidence, these two models were ~four-times more likely to describe the peak urate GCM data than the next best model comprised of FlightPen, TechEntry and DaysAbsent18 (Table 2). After model averaging parameter estimates and standard errors for all candidate models, and intercept-only model, and the global model, FlightPen (β = -1150 ± 533 SE, 90% CI: ¯2030 - ¯275), TechEntry (β = -1490 ± 478 SE, 90% CI: ¯2280 - ¯705), DaysAbsent (β = 1090 ± 586 SE, 90% CI:125 - 2050), and YrsMMFed all remained significant (β = 1100 ± 520 SE, 90% CI:240– 1950, Fig 1C, Table S8). The 90% CI for DaysAbsent18 included zero (Fig 1C, Table S8).

To examine which variables influence the magnitude change in urate GCM levels over 2 hours, we constructed candidate models for prediction of ∆urate GCM. The candidate models for ∆urate GCM levels were similar to those for peak urate GCM levels. The most parsimonious models again contained the variables FlightPen, TechEntry, Days Absent, and YrsMMFed (Table 2). Again DaysAbsent18 was present only in one poorly supported model (AIC_c weight = 0.13, Table 2). The significant model averaged parameters were FlightPen (β = -1380 ± 515 SE, 90% CI: ¯2230 - ¯530), TechEntry (β = -1540 ± 463 SE, 90% CI: ¯2300 - ¯781), and YrsMMFed (β = 1050 ± 500 SE, 90% CI: 222 – 1868, Fig 1C, Table S8).

4. Discussion

4.1. Age, sex, season, and stressor variables and GC response outcomes

4.1.1. Age, Sex, and Season not associated with GC response outcomes in wild condors

Condor age showed a significant interaction with Status when predicting plasma CORT levels when samples from both wild and captive condors were included (β = 49.8 ± 23.2 SE, p = 0.04, Table S7). Via this interaction, Age had a positive effect on wild condor plasma CORT levels but not on captive condor plasma CORT levels. It is possible that Age may also be a predictor for cumulative lead exposure as the influence of age became insignificant after DaysAbsent was added to the model. Age, Sex, and Season were not retained in the model after the first round of model selection (Tables S5 and S6). Sex-specific differences in GC response have been documented in some, but not all, other bird species (Möstl et al., 2005). Since only one of the condors in our study (199, plasma sample only) was actively breeding during the season of sample collection, potential sex related differences in GC levels during breeding were not relevant here. Similarly, our Season variable was meant to capture potential differences in GC response due to seasonal changes in weather that might affect foraging opportunities, and thus alter GCs in circulation. As we found no GC differences in condors due to season, it is possible that artificial food supplementation by condor managers and the relatively mild climate of coastal California may not induce seasonal GC changes in condors, particularly in birds that aren't investing additional energy into breeding attempts.

At least one of the stressor variables (MinSinceHandl, TechEntry, FlightPen) were present in the top models for predicting all urate GCM outcomes, highlighting the importance of accounting for variation in handling protocols in wildlife endocrinology studies (Dickens et al., 2009). Not surprisingly, time since handling start has been shown to positively influence GC levels in other species, including 'baseline' plasma CORT levels (Newman et al., 2017). Indeed, the positive influence of time since an acute stressor event and excreted GCM levels was the basis for our employing repeated measurements of GC for monitoring HPA axis function. We observed an effect of time since handling start (MinSinceHandl) on 1st urate GCM levels in condors (Fig 2, Table S8). However the low explanatory power of MinSinceHandl for first urates ($R^2 = 0.11$), indicate that 1st urate GCM levels are probably sensitive to many different factors that we were not able to account for in our models. For this reason, we conclude that we are not measuring a true baseline GC in urates or plasma and thus cannot reach any conclusions on the influence of lead exposure on baseline GC levels in California condors or the lack thereof in this study.

We recommend use of long-term integrated measures of circulating GC, such as feather GC (Bortolotti et al., 2008), to compare wild condor GC levels outside of handling events. Because California condors are closely monitored and growing feathers often identified and marked when birds are in-hand during routine trap-ups, we are able to estimate the approximate timing of trapping and handling events over the period of feather growth (Glucs et al.2018, in review). Feather corticosterone concentrations have been shown vary over feather growth (Glucs et al. 2018, in review) and pairing hormone measurements with behavioral risk data could provide a

window into impacts of contaminant exposure on GC release outside of captivity and restraint stressors.

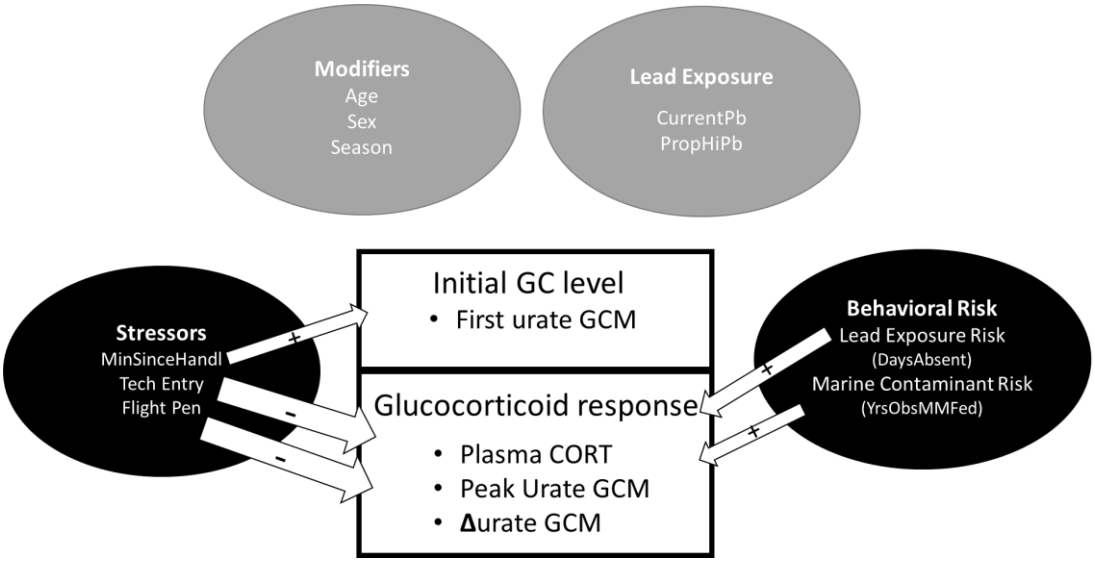


Figure 3. Overview of variables and their influence on California condor glucocorticoid response outcomes. White arrows indicate effect of variable on glucocorticoid response outcomes: first urate glucocorticoid metabolite (GCM) level, plasma corticosterone (CORT) level, peak urate GCM level, and ∆urate GCM level. Width of arrow indicated relative differences in variance explained, and the sign within each arrow indicates positive (+) or negative (-) effect.

Our findings show that differences in trapping procedures are influencing the relative magnitude of GC responses in this large avian species. Interestingly, longer durations of captivity before handling day (FlightPen) as well as longer periods between initial technician entrance to flight pen on day of handling and the start of handling for the individual condor (TechEntry) were both associated with lower peak urate GCM and ∆urate GCM levels in the individuals we studied (Figure 2 and 3). Similarly, Dickens et al. (2009) observed that initial transfer to captivity in wild-caught

chukar (*Alectoris chukar*) resulted in a significant decrease in restraint-induced plasma CORT levels over 3-5 days, with a recovery to pre-captivity levels by day 9. The duration that condors were captive in the flight pen before physical restraint and handling ranged from 1 -10 days, with a median of ~2 days (45 hr), and so perhaps condors exhibit a similar acclimation period, with stress-induced GCM values decreasing as time in flight pen increases. The negative influence of TechEntry has not been evaluated in GC literature, as condor capture is likely one of the few trapping protocols that regularly necessitates multi-step capture (passive trapping from wild before manual trapping in pen) for multiple birds per day. We can however offer a three possible explanations for the negative relationship between TechEntry and stress induced GCM: i) habituation to repeated technician entries before capture, ii) additive stressors, and iii) rapid negative feedback of GCs on the HPA axis. Condors with a longer period between first technician entry of day and handling start were likely witness to more tech entries and more condor captures, than birds with shorter periods since technician entry. There is evidence for habituation of GC response to capture and restraint following longer term stressors such as ecotourism pressure (Walker et al., 2006). However, habituation to repeated chasing and restraint in shorter time frames (days) is often behavioral and not reflected in physiological measurements such as GC levels (Conde-Sieira et al., 2018; Jones et al., 2000). In regards to the potential for additive stress, it is possible restraint closely following the initial trapping to flight pen, or the first technician entry of the day may compound the stress response, resulting in higher GC elevation when these multiple stressors are occurring closer together. Moreover, condors with longer periods of time to physiologically recover from the first human intrusion may have more

attenuated hormonal responses to the restraint stress. Finally, it has been shown that steroid specific neuronal membrane receptors may allow for more rapid action of glucocorticoids on brain cells, causing rapid behavioral changes (milliseconds to minutes) and potentially allowing for rapid negative feedback on the parvocellular neurons in the hypothalamus (Orchinik et al., 1994). These neural receptors have been identified in birds(Breuner and Orchinik, 2009) and rapid induction of negative feedback mechanisms during initial tech entry to flight pen might account for the observed suppression of peak levels of GCM over time since the initial stressor of TechEntry . In general the body of literature regarding experimentally induced, repeated, acute stress responses within short time-frames (1 day) is limited due to human and animal welfare considerations (see Conde-Sieira et al., 2018). Future studies should account for these pre-restraint stressors before testing other modifiers of the GC response in birds, and aim to limit pre-restraint stressors when feasible.

4.2. Insufficiency of blood lead to characterize lead exposure history

While lead has a half-life of about 2 weeks in the blood (Kaushal et al., 1996), the metal's effects can be long lasting in several organ systems, including the endocrine system and specifically the GC stress response (Cory-Slechta et al., 2008). Hypotheses for how lead can increase the amount of circulating GCs generally include impairment of the negative feedback of circulating GCs on the hypothalamic pituitary adrenal (HPA) axis which closely controls GC levels in the blood (Rossi-George et al., 2009). This dynamic and sometimes permanent impairment potentially results from failure of parvocellular neurons in the hypothalamus to receive this negative feedback signal and reduce secretion of the stimulatory corticotropin-releasing hormone, a plausible mechanism for HPA-axis

dysfunction since lead is particularly neurotoxic (White et al., 2007). Increased duration of GC elevation after a stressor is observed in children with blood levels of 10 µg/dL (Gump et al., 2008), which is at least four-fold less than what is typically experienced by a condor during a lead exposure event (Finkelstein, 2012).

The higher peak urate GCM levels observed in wild condors compared to non-lead exposed captive condors is consistent with the hypothesis that lead exposure is heightening the stress response in condors, but none of the blood lead measures that we evaluated were influential in predicting condor stress response within the wild population. While this population of wild condors has arguably one of the most extensive lead exposure history records of any wild species with biannual to yearly blood lead data available for most individuals (USFWS, 2013), blood lead measurements are limited in reflecting lead exposure because of the relatively short half-life of lead in condor blood (~2 weeks;(Finkelstein et al., 2012; Rideout et al., 2012). As such, biannual blood lead testing in California condors has been shown to poorly reflect a bird's exposure history by i) consistently underestimating by 1.4 -14.4 fold the peak blood lead level for an exposure event proximal to when the blood was collected, and ii) underestimating the annual number of exposure events exposures (Finkelstein et al., 2012; Rideout et al., 2012). Prior studies comparing blood lead levels and glucocorticoids in birds have assessed species with chronic routes of exposure, such as food contamination through atmospheric deposition, water, or soil (e.g. Baos et al., 2006; Meillère et al., 2016), rather than the episodic and acute exposures condors experience when they ingest lead-based ammunition fragments (Finkelstein et al., 2010). Thus, similar to our prior work assessing variables affecting condor survival (Bakker et al 2016), we have shown that behaviors associated with

increased lead risk (e.g., days absent from management area or DaysAbsent) are better indicators of lead exposure risk than blood lead measurements.

4.3. Behavioral variables and elevated GC response

As the central coast flock of California condors has aged and multiplied, condors have grown to rely less on proffered, lead-free food sources and spend more time away from the management area (Bakker et al., 2016). As long as spent lead ammunition is present on the landscape, even in as few as 5% of available carcasses, exposure risk will remain high for California condors that forage for their meals (Finkelstein et al., 2012). This flock of condors has also come to regularly utilize marine mammal carcasses such as California sea lions (*Zalophus californianus*) along the coastal margin of their range (Burnett et al., 2013; Kurle et al., 2016). Persistent organic pollutants found in condors and their marine mammal food sources, such as methyl-mercury, p,p′-DDE (a major metabolite of the chlorinated pesticide DDT), PCBs, and polybrominated diphenyl ethers, have previously been documented to have endocrine-disrupting effects in vertebrates (Chen et al., 2017; Herring et al., 2012; Hoffmann and Kloas, 2016; von Hippel et al., 2018). Specifically, PCBs have been frequently associated with altered GC release in seabirds but there is a significant degree of variation in effect they have by species and by sex, with some species even experiencing attenuated GC release in response to stress (S. Tartu et al., 2015a). DaysAbsent and YrsMMFed were associated with positive effects on our measures of stress-induced GC release: plasma CORT, peak GCM levels, and ∆urate GCM levels (Figure 1A, 1C, and 1D). Both DaysAbsent and YrsMMFed variables have been associated with exposure risk for lead and persistent organic pollutants respectively (Bakker et al., 2016, Kurle et

al. 2016). While we acknowledge these behaviors are not perfect proxies for cumulative exposure, they provide information over a long time period at a finer time scale (daily records vs. biannual blood measurements) and may provide a more informative indicator of relative exposure among wild condors. Plasma PCB levels recorded in marine foraging California condors (range ∑PCBs: 7- 546 ng/g ww; Kurle et al., 2016) are within the range of levels detected in seabirds showing elevated stress-induced GC release (range ∑PCBs: 10- 80 ng/g ww, Tartu et al., 2014) and associated delays in egg hatching (range ∑PCBs: 5.8- 560 ng/g ww,Tartu et al., 2015). The degree of GC alteration in condors attributable to our behavioral exposure risk variables has been implicated to cause reductions in reproductive fitness and survival in other avian species (Blas et al., 2007; Sabrina Tartu et al., 2015). While it remains unknown in this case whether GC elevation from contaminant exposures is impacting wild condor health, this study lays the groundwork for understanding this potential route of sub-lethal effects from lead, PCBs, and other persistent organic pollutants.

5. Conclusion

Lead poisoning of wildlife is a global problem and our results indicate that condors exhibiting behaviors associated with higher lead exposure risk may be experiencing a heightened stress response. The potential for sub-lethal effects of lead and other contaminants has important implications for the recovery of this critically endangered species, and can provide insight for other more widespread species that utilize similar food resources. We also illustrate the challenges with understanding contaminant exposure in a wild species as condors are exposed to

multiple pollutants. We show that persistent organic pollutants (e.g., PCBs) are also associated with a heightened stress response in condors, potentially compounding the effects of lead. Importantly, our work underscores the need to account for captivity and pre-handing stressors when investigating GC responses in wild-caught individuals. The variation in these stressors between individuals can mask potential effects of contaminants if not properly documented controlled for. Ultimately we illustrate that sub-lethal contaminant exposure is impacting the stress response of a long-lived avian scavenger, a group of species for which contaminant exposure is of major concern due to their feeding behaviors.

References

Arnold, T.W., 2010. Uninformative Parameters and Model Selection Using Akaike' s Information Criterion. J. Wildl. Manage. 74, 1175–1178. doi:10.2193/2009-367

Bakker, V.J., Smith, D.R., Copeland, H., Brandt, J., Wolstenholme, R., Burnett, J., Kirkland, S., Finkelstein, M.E., 2016. Effects of lead exposure, flock behavior, and management actions on the survival of California condors (*Gymnogyps californianus*). Ecohealth 1–14. doi:10.1007/s10393-015-1096-2

Baos, R., Blas, J., Bortolotti, G.R., Marchant, T. a., Hiraldo, F., 2006. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (*Ciconia ciconia*) exposed to heavy metal and arsenic contamination. Environ. Health Perspect. 114, 1497–1501. doi:10.1289/ehp.9099

Bellinger, D.C., Burger, J., Cade, T.J., Cory-Slechta, D.A., Finkelstein, M., Hu, H., Kosnett, M., Landrigan, P.J., Lanphear, B., Pokras, M.A., Redig, P.T., Rideout, B.A., Silbergeld, E., Wright, R., Smith, D.R., 2013. Health risks from lead-based ammunition in the environment. Environ. Health Perspect. 121. doi:10.1289/ehp.1306945

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. Proc. Natl. Acad. Sci. U. S. A. 104, 8880–8884.

Bortolotti, G.R., Marchant, T. a., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct. Ecol. 22, 494–500. doi:10.1111/j.1365-2435.2008.01387.x

Breuner, C.W., Orchinik, M., 2009. Pharmacological characterization of intracellular, membrane, and plasma binding sites for corticosterone in house sparrows. Gen. Comp. Endocrinol. 163, 214–224. doi:10.1016/j.ygcen.2009.01.027

Burnett, L.J., Sorenson, K.J., Brandt, J., Sandhaus, E.A., Ciani, D., Clark, M., David, C., Theule, J., Kasielke, S., Risebrough, R.W., 2013. Eggshell thinning and depressed hatching success of California condors reintroduced to central California. Condor 115, 477–491. doi:10.1525/cond.2013.110150

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Interference: A Practical Information-Theoretic Approach, 2nd ed. Springer-Verlag, New York.

Cade, T.J., 2007. Exposure of California condors to lead from spent ammunition. J. Wildl. Manage. 71, 2125. doi:10.2193/2007-084

Chen, L., Wang, X., Zhang, X., Lam, P.K.S., Guo, Y., Lam, J.C.W., Zhou, B., 2017. Transgenerational endocrine disruption and neurotoxicity in zebrafish larvae after parental exposure to binary mixtures of decabromodiphenyl ether (BDE-209) and lead. Environ. Pollut. 230, 96–106. doi:10.1016/j.envpol.2017.06.053

Church, M.E., Gwiazda, R., Risebrough, R.W., Sorenson, K., Chamberlain, C.P., Farry, S., Heinrich, W., Rideout, B. a, Smith, D.R., 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environ. Sci. Technol. 40, 6143–50.

Conde-Sieira, M., Valente, L.M.P., Hernández-Pérez, J., Soengas, J.L., Míguez, J.M., Gesto, M., 2018. Short-term exposure to repeated chasing stress does not induce habituation in Senegalese sole (*Solea senegalensis*). Aquaculture 487, 32–40. doi:10.1016/j.aquaculture.2018.01.003

Cory-Slechta, D.A., Virgolini, M.B., Rossi-George, A., Thiruchelvam, M., Lisek, R., Weston, D., 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin. Pharmacol. Toxicol. 102, 218–27. doi:10.1111/j.1742-7843.2007.00189.x

Dickens, M.J., Earle, K. a, Romero, L.M., 2009. Initial transference of wild birds to captivity alters stress physiology. Gen. Comp. Endocrinol. 160, 76–83. doi:10.1016/j.ygcen.2008.10.023

Ellenberg, U., Setiawan, A.N., Cree, A., Houston, D.M., Seddon, P.J., 2007. Elevated hormonal stress response and reduced reproductive output in Yellow-eyed penguins exposed to unregulated tourism. Gen. Comp. Endocrinol. 152, 54–63. doi:10.1016/j.ygcen.2007.02.022

Finkelstein, M., Kuspa, Z., Snyder, N.F., Schmitt, N.J., 2015. California Condor Species Account, Birds of North America.

Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D.R., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc. Natl. Acad. Sci. U. S. A. 109, 11449–54. doi:10.1073/pnas.1203141109

Finkelstein, M.E., George, D., Scherbinski, S., Gwiazda, R., Johnson, M., Burnett, J., Brandt, J., Lawrey, S., Pessier, a P., Clark, M., Wynne, J., Grantham, J., Smith, D.R., 2010. Feather lead concentrations and (207)Pb/(206)Pb ratios reveal lead exposure history of California Condors (Gymnogyps californianus). Environ. Sci. Technol. 44, 2639–47. doi:10.1021/es903176w

Fortin, M.C., Cory-Slechta, D.A., Ohman-Strickland, P., Yanger, T.S., Todd, A.C., Moynihan, J., Walton, J., Brooks, A., Fiedler, N., Nwankwo, C., Cory, D.A., Johnson, W., 2012. Increased lead biomarker levels are associated with changes in hormonal response to stress in occupationally exposed male participants. Environ. Health Perspect. 120, 278–283.

Gelman, A., 2008. Scaling regression inputs by dividing two standard deviations. Stat. Med. 27, 2865–2873. doi:10.1002/sim

Gump, B.B., Stewart, P., Reihman, J., Lonky, E., Darvill, T., Patrick, J., Granger, D.A., Parsons, P.J., 2008. Children's low-level prenatal and postnatal blood lead exposure and adrenocortical responses to acute stress in children. Environ. Health Perspect. 116, 249–255. doi:10.1289/ehp.l0391

Herring, G., Ackerman, J.T., Herzog, M.P., 2012. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ. Sci. Technol. 46, 6339–6346. doi:10.1021/es300668c

Herring, G., Gawlik, D.E., 2009. Stability of avian fecal corticosterone metabolite levels in frozen avian feces. J. Wildl. Manage. 73, 1010–1013. doi:10.2193/2008-398

Hirschenhauser, K., Spreitzer, K., Lepschy, M., Kotrschal, K., Möstl, E., 2012. Excreted corticosterone metabolites differ between two galliform species, Japanese Quail and Chicken, between sexes and between urine and faecal parts of droppings. J. Ornithol. 153, 1179–1188. doi:10.1007/s10336-012-0848-9

Hoffmann, F., Kloas, W., 2016. p,p′-Dichlordiphenyldichloroethylene (p,p′-DDE) can elicit antiandrogenic and estrogenic modes of action in the amphibian *Xenopus laevis*. Physiol. Behav. 167, 172–178. doi:10.1016/j.physbeh.2016.09.012

Hunt, W., 2012. Implications of sublethal lead exposure in avian scavengers. J. Raptor Res. 46, 389–393.

Johnson, C.K., Kelly, T.R., Rideout, B.A., 2013. Lead in ammunition: A persistent threat to health and conservation. Ecohealth 10, 455–464. doi:10.1007/s10393-013-0896-5

Jones, B.C., Smith, A.D., Bebus, S.E., Schoech, S.J., 2016. Two seconds is all it takes: European starlings (*Sturnus vulgaris*) increase levels of circulating glucocorticoids after witnessing a brief raptor attack. Horm. Behav. 78, 72–78. doi:10.1016/j.yhbeh.2015.10.017

Jones, R.B., Satterlee, D.G., Waddington, D., Cadd, G.G., 2000. Effects of repeated restraint in Japanese quail genetically selected for contrasting adrenocortical responses. Physiol. Behav. 69, 317–324. doi:10.1016/S0031-9384(00)00204-3

Kaushal, D., Garg, M.L., Bansal, M.R., Bansal, M.P., 1996. Biokinetics of lead in various mouse organs using radiotracer technique. Biol. Trace Elem. Res. 53, 249–260. doi:10.1007/BF02784561

Kurle, C.M., Bakker, V.J., Copeland, H., Burnett, J., Jones Scherbinski, J., Brandt, J., Finkelstein, M.E., 2016. Terrestrial scavenging of marine mammals: cross-ecosystem contaminant transfer and potential risks to endangered California condors (*Gymnogyps californianus*). Environ. Sci. Technol. acs.est.6b01990. doi:10.1021/acs.est.6b01990

Lukacs, P.M., Burnham, K.P., Anderson, D.R., 2010. Model selection bias and Freedman ' s paradox 117–125. doi:10.1007/s10463-009-0234-4

Mazerolle, M.J., 2017. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R Packag. version 2.1-1. URL https://cran.r-project.org/package=AICcmodavg

Meillère, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., Angelier, F., 2016. Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*). Sci. Total Environ. 566–567, 93–101. doi:10.1016/j.scitotenv.2016.05.014

Möstl, E., Rettenbacher, S., Palme, R., 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Ann. N. Y. Acad. Sci. 1046, 17–34. doi:10.1196/annals.1343.004

Newman, A.E.M., Hess, H., Woodworth, B.K., Norris, D.R., 2017. Time as tyrant: the minute, hour and day make a difference for corticosterone concentrations in wild nestlings. Gen. Comp. Endocrinol. 250, 80–84. doi:10.1016/j.ygcen.2017.05.022

Orchinik, M., Moore, F.L., Rose, J.D., 1994. Mechanistic and functional studies of rapid corticosteroid actions. Ann. N. Y. Acad. Sci. 746, 101-112-114. doi:10.1111/j.1749-6632.1994.tb39219.x

Owen, J.C., Nakamura, A., Coon, C.A., Martin, L.B., 2012. The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). Vet. Res. 43, 34. doi:10.1186/1297-9716-43-34

Pain, D., 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. Ingestion Lead from Spent Ammunit. Implic. Wildl. Humans 99–118. doi:10.4080/ilsa.2009.0108

R Core Development Team, 2011. R: A Language and Environment for Statistical Computing.

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., Palme, R., 2004. Measurement of corticosterone metabolites in chicken droppings. Br. Poult. Sci. 45, 704–11. doi:10.1080/00071660400006156

Rideout, B.A., Stalis, I., Papendick, R., Pessier, A., Puschner, B., Finkelstein, M.E., Smith, D.R., Johnson, M., Mace, M., Stroud, R., Brandt, J., Burnett, J., Parish, C., Petterson, J., Witte, C., Stringfield, C., Orr, K., Zuba, J., Wallace, M., Grantham, J., 2012. Patterns of mortality in free-ranging California Condors (*Gymnogyps californianus*). J. Wildl. Dis. 48, 95–112.

Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 73–79. doi:10.1016/j.cbpb.2004.11.004

Rossi-George, A., Virgolini, M.B., Weston, D., Cory-Slechta, D.A., 2009. Alterations in glucocorticoid negative feedback following maternal Pb, prenatal stress and the combination: a potential biological unifying mechanism for their corresponding disease profiles. Toxicol. Appl. Pharmacol. 234, 117–27. doi:10.1016/j.taap.2008.10.003

Schoenle, L.A., Dudek, A.M., Moore, I.T., Bonier, F., 2017. Hormones and Behavior Red-winged blackbirds (*Agelaius phoeniceus*) with higher baseline glucocorticoids also invest less in incubation and clutch mass. Horm. Behav. 90, 1–7. doi:10.1016/j.yhbeh.2017.02.002

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? Gen. Comp. Endocrinol. 166, 614–9. doi:10.1016/j.ygcen.2009.12.017

Tartu, S., Angelier, F., Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Gabrielsen, G.W., Verboven, N., Verreault, J., Labadie, P., Budzinski, H., Wingfield, J.C., Chastel, O., 2015a. Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species. Environ. Pollut. 197, 173–180. doi:10.1016/j.envpol.2014.12.007

Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated: Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476–477, 553–560. doi:10.1016/j.scitotenv.2014.01.060

Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015b. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. doi:10.1016/j.scitotenv.2014.10.008

Tartu, S., Lendvai, Á.Z., Blévin, P., Herzke, D., Bustamante, P., Moe, B., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015. Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*). Gen. Comp. Endocrinol. 219, 165–172. doi:10.1016/j.ygcen.2014.12.018

Tempel, D., Gutiérrez, R., 2004. Factors related to fecal corticosterone levels in California spotted owls: implications for assessing chronic stress. Conserv. Biol. 18, 538–547. doi:10.1111/j.1523-1739.2004.00372.x

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046, 54–74. doi:10.1196/annals.1343.006

United States Fish and Wildlife Service, 2013. California Condor 5-Year Review : Summary and Evaluation.

Virgolini, M.B., Bauter, M.R., Weston, D.D., Cory-Slechta, D. a, 2006. Permanent alterations in stress responsivity in female offspring subjected to combined maternal lead exposure and/or stress. Neurotoxicology 27, 11–21. doi:10.1016/j.neuro.2005.05.012

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: Health implications for indigenous residents. Environ. Pollut. 234, 279–287. doi:10.1016/j.envpol.2017.11.054

Walker, B.G., Dee Boersma, P., Wingfield, J.C., 2006. Habituation of adult Magellanic Penguins to human visitation as expressed through behavior and corticosterone secretion. Conserv. Biol. 20, 146–154. doi:10.1111/j.1523-1739.2005.00271.x

Washburn, B.E., Morris, D.L., Millspaugh, J.J., Faaborg, J., Schulz, J.H., 2002. Using a commercially available radioimmunoassay To quantify corticosterone in avian plasma. Condor 104, 558–563.

White, L.D., Cory-Slechta, D. a, Gilbert, M.E., Tiffany-Castiglioni, E., Zawia, N.H., Virgolini, M., Rossi-George, A., Lasley, S.M., Qian, Y.C., Basha, M.R., 2007. New and evolving concepts in the neurotoxicology of lead. Toxicol. Appl. Pharmacol. 225, 1–27. doi:10.1016/j.taap.2007.08.001

Wilcoxen, T.E., Boughton, R.K., Bridge, E.S., Rensel, M. A, Schoech, S.J., 2011. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. Gen. Comp. Endocrinol. 173, 461–6. doi:10.1016/j.ygcen.2011.07.007

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42, 600–9. doi:10.1093/icb/42.3.600

CHAPTER 5: CONCLUSION

When it comes to hormone measurement in a previously untested species, the choice of immunoassay kit matters. I determined that results from two different corticosterone immunoassay kits were not comparable and that the MP Biomedicals radioimmunoassay was appropriate for use across condor plasma, urates, and feather extracts. Additionally, the defined trapping and physical restraint that take place during regular condor health checks produced a significant elevation in glucocorticoid metabolites in urates, and this glucocorticoid response to stress was higher in wild than in captive condors. Within the wild condor flock, variation in pre-handling stressor events such as time in flight pen and time since initial rech entry explained ~25% of the observed variance in glucocorticoid response magnitude. After accounting fot the impact of these stressors on wild condors, behaviors associated with increased risk of lead and persistent organic pollutants were both positively associated with glucocorticoid response outcomes.

My work, illustrating that lead and other environmental contaminant exposures are associated with an elevated hormonal stress response in California condors, is a novel discovery with significant implication for the long-term sustainability and survival of condors in the wild. More broadly, my work informs concerns over the risk from environmental contaminants suffered by long-lived avian scavengers, who are considered a globally threatened bird group. The world's vulture species are experiencing population crises at a disproportionate rate, primarily due to dietary toxins (Buechley and Şekercioğlu, 2016). Furthermore, lead poisoning from the use of ammunition is a threat for scavenging wildlife across multiple continents (Carneiro et al., 2016; Madry et al., 2015; Naidoo et al., 2017).

Thus, my work on the intensively monitored California condor can inform studies of other threatened and ecologically important species that are not as well-studied as the condor. Importantly, my results that indicate the compounding effects of multiple threats, like those experienced by condors feeding on contaminated terrestrial (lead) and marine food sources (PCBs), on hormonal function underscore the fact that wild species are often exposed to multiple contaminants throughout their life.

The relative effects of pre-handling stressor trapping events on the magnitude of glucocorticoid stress responses measured in urates was much greater than the estimated effects of contaminant exposure, as one would expect, illustrating that future studies must carefully standardize and quantify the parameters of trapping and handling to account for their influence on the stress response. In future studies aiming to detect alterations to the glucocorticoid stress response in wild species, I recommend highly controlled stressor experiments that either limit or closely replicate pre-experiment stress for all individuals as much as is logistically feasible. Specific to my study, glucocorticoid comparisons between condors with and without marine contaminant exposure may help to untangle the relative influence of lead and marine contaminant exposure on a condor's stress response. Additional work is needed to determine whether the documented positive association between lead risk and a condor's stress response is also affecting a condor's survival and reproduction.

APPENDICES

Supplemental Information for Chapter 2

Appendix A.

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

1) During routine health monitoring of free-flying California condors (typically in the spring and fall), a whole blood sample (1 – 3 mL) from the tibiotarsal vein is collected into low-lead Vacutainers with EDTA anti-coagulant (Fisher Scientific, Pittsburgh, PA) using a 19 or 21-gauge catheter, as previously described (Church et al., 2006; Finkelstein et al., 2010). Whole blood samples are stored at -20º C until analysis.
2) When condors are handled for blood sampling, birds are examined for growing primary feathers. Growing feathers are measured (length, follicle to tip) and marked to facilitate identification for potential future collection, typically by cutting a 3-5 mm notch in the leading edge of the primary feather vane. Upon recapture for a subsequent health check, previously marked fully grown feathers are identified and an ~2 cm deep margin of trailing vane is cut along the entire rachis and stored in polyethylene bags at room temperature until processing for analysis. At UCSC feather samples are cut into ~2 cm wide sections (perpendicular to rachis) for individual analysis as described in Finkelstein et al. (2010).
3) If a condor is lead poisoned (blood lead >35 µg/dL), the condor is typically transferred to the Gottlieb Animal Health and Conservation Center (LA Zoo, California, USA) for chelation treatment and observation, and growing primary feathers are marked and

measured for potential collection and analysis, as described above. As the principal cause of lead exposure in condors is ingestion of a lead item, whole body radiographs are performed to detect the presence of radio-opaque objects within the bird's digestive tract and assess the need for surgical or other intervention. Radio-opaque objects may be surgically removed or recovered following induced regurgitation.

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

Sample collection details for California condors 286, 375, 401.

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

Condor 375. On 26 March 2009 condor 375 was captured by VWS field biologists for routine lead exposure monitoring. A blood sample identified the bird as lead poisoned (blood lead value of "High", LeadCare II field collection kit) and she was transported to the LA Zoo for chelation treatment. Radiographs revealed three birdshot pellets embedded in the bird's tissue. One pellet was surgically removed. The condor recovered and a growing primary

feather vane sample was collected (total vane length = 21.4 cm) on 1 May 2009 prior to release back into the wild.

Condor 401. On 12 April 2009 condor 401 was captured by Pinnacles National Park (PNP, formally Pinnacles National Monument) biologists for routine lead exposure monitoring. A blood sample identified the bird as lead exposed (>10µg/dL, Cade, 2007); field value 11µg/dL, Lead Care II field collection kit), but below the 35 µg/dL blood lead level threshold for chelation treatment. A growing primary feather was identified and marked for later collection.

On 27 May 2009 condor 401 was recaptured by PNP biologists for routine lead exposure monitoring. A blood sample identified the bird as lead exposed (field value 13µg/dL, Lead Care II field collection kit) and the trailing vane of the previously marked primary feather was collected (total vane length = 39.3cm).

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

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

marked feather was subsequently collected on 18 May 2011 after the feather was fully grown (total vane length = 39.9cm).

Appendix B.

Condor feather growth timeline.

As we have noted previously (Finkelstein et al., 2010), estimation of a feather growth timeline depends on accurate feather length measurements, though obtaining accurate calamus and total feather length measurements from restrained live birds can be challenging. Further, the time lag between when a growing vane segment becomes isolated from the blood supply in the proximal calamus region to when it emerges from the calamus sheath and becomes available for sampling is unknown. For condors 375 and 401 (27 May 2009 collection), whose feathers were still growing when collected, we estimated this time lag to be ~ 6 days; i.e., the newly grown proximal feather vane segment may reflect lead in the bird's blood from ~6 days before this vane segment emerged from the calamus sheath and was collected. Our time lag adjustment is supported by the observation that the lead isotopic composition from the blood samples collected during feather growth are not measurably different than the corresponding feather sections for these dates (Fig. 2B).

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

composition from the blood sample collected on 4 March 2009 was measurably indistinguishable from the corresponding feather section for this date (Fig. 2B), supporting our adjustments to condor 286's feather growth rate.

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

Table A1. Timeline of case study-related events for condors 286, 375, and 401 from January 2009 through May 2011. Sample collection was performed by Ventana Wildlife Society (VWS), Pinnacles National Park (PNP), the Gottlieb Animal Health and Conservation Center (LAZ), and The Wildlife Disease Laboratories at the Institute for Conservation Research San Diego Zoo Global (SDZ). Field blood lead levels were measured using a LeadCare II field collection kit, while additional samples were collected simultaneously for more precise measurements of blood lead concentrations at the University of California, Santa Cruz (UCSC) unless otherwise noted.

Date	Condor ID	Event/Action
January 2009	286,375,401	Estimated date of shooting event(s).
28 January 2009	286	Behavioral change noted by PNP staff biologists.
4 March 2009	286	Trapped at VWS field site due to observed behavioral change, field blood lead level “High”, UCSC blood lead level 155 µg/dL.
5 March 2009	286	Transported to LAZ for treatment, 10 embedded birdshot pellets identified via radiograph.
26 March 2009	375	Trapped by VWS for routine health check, field blood lead level “High”, UCSC blood lead level 180 µg/dL, transported to LAZ for treatment, three embedded birdshot pellets identified via radiograph.
12 April 2009	401	Trapped by PNP for routine health check, field blood lead level 11 µg/dL, UCSC blood lead level 16.6 µg/dL, growing right primary feather #3 (RP3) measured and marked. Bird re-released to wild.
20 April 2009	375	One birdshot pellet surgically removed.
1 May 2009	375	Re-released to wild by VWS post-treatment. Prior to release, blood and growing feather collected by VWS, UCSC blood lead level 34.7 µg/dL.
11 May 2009	286	Died of lead toxicosis, five embedded birdshot pellets, growing feather, bone, kidney, and liver samples collected by necropsy staff at the SDZ.
27 May 2009	401	Trapped by PNP for routine health check, field blood lead level 13 µg/dL, UCSC blood lead level 18.0 µg/dL, growing feather (RP3) collected. Re-released to wild.
30 October 2009	401	Trapped by VWS for routine health check, field blood lead level “High”, Louisiana Animal Disease Diagnostics Laboratory blood lead level 86 µg/dL.
1 November 2009	401	Transported to LAZ for treatment, four embedded birdshot pellets identified via radiograph.
6 November 2009	401	One birdshot pellet surgically removed.
19 November 2009	401	Re-released to wild by VWS post-treatment.

Date	Condor ID	Event/Action
11 May 2010	401	Trapped by PNP for routine health check, field blood lead level 22 µg/dL, UCSC blood lead level 25.7 µg/dL, growing feather (RP8) measured and notched.
21 June 2010	401	Trapped by VWS, field blood lead level “High”, UCSC blood lead level 556 µg/dL, transported to LAZ for treatment. Three embedded birdshot pellets in wing and one buckshot shot pellet in digestive tract identified via radiograph (Fig. A1).
22 June 2010	401	One birdshot pellet surgically removed.
26 June 2010	401	Ingested buckshot pellet collected from regurgitated casting by LAZ staff.
8 September 2010	401	Transferred to PNP and monitored in captivity.
6 October 2010	401	Re-released to wild by PNP.
18 May 2011	401	Trapped by PNP for routine health check, field blood lead level “High,” fully grown feather (RP8) collected.
19 May 2011	401	Transported to LAZ for treatment.
7 June 2011	401	Re-released to wild by PNP post treatment.

Table A2. Sample collection information, lead concentrations, $^{207}Pb/^{206}Pb$ ratios, and $^{208}Pb/^{206}Pb$ ratios of tissues collected from condors 286, 375, and 401. Also shown are the $^{207}Pb/^{206}Pb$ ratios and $^{208}Pb/^{206}Pb$ ratios of the recovered birdshot and buckshot.

Condor ID #	Sample	Sample collection date	Feather segment # (segment length)[a]	[Pb][b]	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$
286	Birdshot	5/17/2009	--	--	0.8187	2.0178
286	Birdshot	5/17/2009	--	--	0.8183	2.0183
286	Birdshot	5/17/2009	--	--	0.8194	2.0203
286	Birdshot	5/17/2009	--	--	0.8194	2.0191
286	Birdshot	5/17/2009	--	--	0.8189	2.0179
286	Blood	3/4/2009	--	155	0.8194	2.0150
286	Liver	5/11/2009	--	4.07	0.8190	2.0129
286	Kidney	5/11/2009	--	1.72	0.8167	2.0158
286	Bone	5/11/2009	--	21.0	0.8185	2.0164
286	Feather	Post-mortem	1 (2.0)	0.33	0.8231	2.0327
286	Feather	Post-mortem	3 (2.0)	0.74	0.8220	2.0299
286	Feather	Post-mortem	4 (2.0)	0.76	0.8219	2.0233
286	Feather	Post-mortem	5 (2.0)	0.74	0.8236	2.0320
286	Feather	Post-mortem	6 (2.0)	0.70	0.8247	2.0316
286	Feather	Post-mortem	7 (2.0)	0.69	0.8247	2.0355
286	Feather	Post-mortem	8 (2.0)	0.92	0.8231	2.0317
286	Feather	Post-mortem	9 (2.0)	3.30	0.8200	2.0252
286	Feather	Post-mortem	11 (2.0)	19.9	0.8179	2.0205
286	Feather	Post-mortem	13 (2.0)	38.5	0.8187	2.0242
286	Feather	Post-mortem	14 (2.0)	26.0	0.8191	2.0180
286	Feather	Post-mortem	15 (2.0)	12.3	0.8188	2.0217
286	Feather	Post-mortem	16 (2.5)	0.93	0.8210	2.0203
375	Birdshot	4/20/2009	--	--	0.8184	2.0167
375	Blood	3/26/2009	--	180	0.8225	2.0178
375	Blood	5/1/2009	--	34.7	0.8248	2.0229
375	Feather	5/1/2009	1 (2.0)	12.7	0.8217	2.0167
375	Feather	5/1/2009	2 (2.0)	9.93	0.8231	2.0213
375	Feather	5/1/2009	3 (2.0)	9.07	0.8231	2.0206
375	Feather	5/1/2009	4 (2.0)	8.38	0.8236	2.0225
375	Feather	5/1/2009	5 (2.0)	7.78	0.8238	2.0244
375	Feather	5/1/2009	6 (2.0)	5.04	0.8235	2.0235
375	Feather	5/1/2009	7 (2.0)	3.57	0.8262	2.0270
375	Feather	5/1/2009	8 (2.0)	1.63	0.8246	2.0251
375	Feather	5/1/2009	9 (2.0)	0.73	0.8264	2.0272
375	Feather	5/1/2009	10 (2.0)	0.49	0.8271	2.0239
375	Feather	5/1/2009	11 (1.4)	0.48	0.8264	2.0242

Condor ID #	Sample	Sample collection date	Feather segment # (segment length)[a]	[Pb][b]	$^{207}Pb/^{206}Pb$	$^{208}Pb/^{206}Pb$
401	Birdshot	11/6/2009	--	--	0.8191	2.0205
401	Birdshot	6/22/2010	--	--	0.8182	2.0185
401	Buckshot	6/26/2010	--	--	0.8122	1.9982
401	Blood	4/12/2009	--	16.6	0.8166	2.0101
401	Blood	5/27/2009	--	18.0	0.8161	2.0124
401	Blood	5/11/2010	--	25.7	0.8331	2.0470
401	Blood	6/21/2010	--	556	0.8130	2.0049
401	Feather	4/12/2009	1 (3.8)	1.45	0.8189	2.0188
401	Feather	5/27/2009	2+3 (4.0)	1.18	0.8208	2.0236
401	Feather	5/27/2009	4 (2.0)	1.05	0.8194	2.0177
401	Feather	5/27/2009	5 (2.0)	0.89	0.8199	2.0170
401	Feather	5/27/2009	7 (2.0)	0.85	0.8210	2.0211
401	Feather	5/27/2009	9 (2.0)	0.78	0.8184	2.0164
401	Feather	5/27/2009	11 (2.0)	0.67	0.8180	2.0160
401	Feather	5/27/2009	13 (2.0)	0.54	0.8185	2.0168
401	Feather	5/27/2009	15 (2.0)	0.53	0.8180	2.0182
401	Feather	5/27/2009	17 (2.0)	0.55	0.8179	2.0189
401	Feather	5/27/2009	18+19 (3.5)	0.53	0.8181	2.0187
401	Feather	5/18/2011	1 (1.3)	3.36	0.8631	2.0908
401	Feather	5/18/2011	3 (1.9)	1.76	0.8528	2.0783
401	Feather	5/18/2011	6 (2.1)	1.41	0.8449	2.0668
401	Feather	5/18/2011	9 (2.1)	1.03	0.8401	2.0596
401	Feather	5/18/2011	12 (2.0)	1.00	0.8365	2.0533
401	Feather	5/18/2011	15 (2.0)	15.1	0.8162	2.0147
401	Feather	5/18/2011	18 (1.9)	53.7	0.8161	2.0010
401	Feather	5/18/2011	21 (2.0)	53.6	0.8142	2.0042

[a] Feather vane segments ordered distal (oldest) → proximal (newest) (segment length in cm).

[b] Blood Pb concentrations in µg/dL, feather and tissue Pb concentrations in µg/g dry weight.

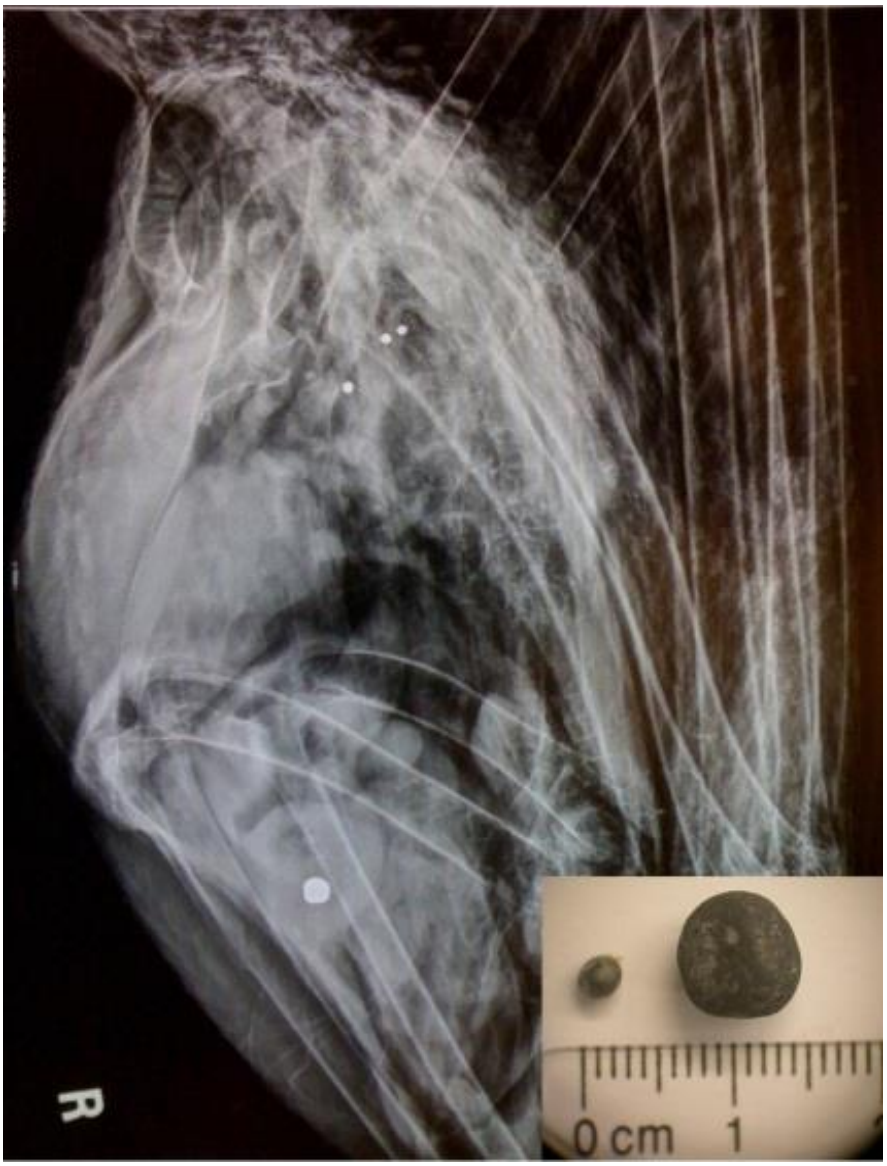
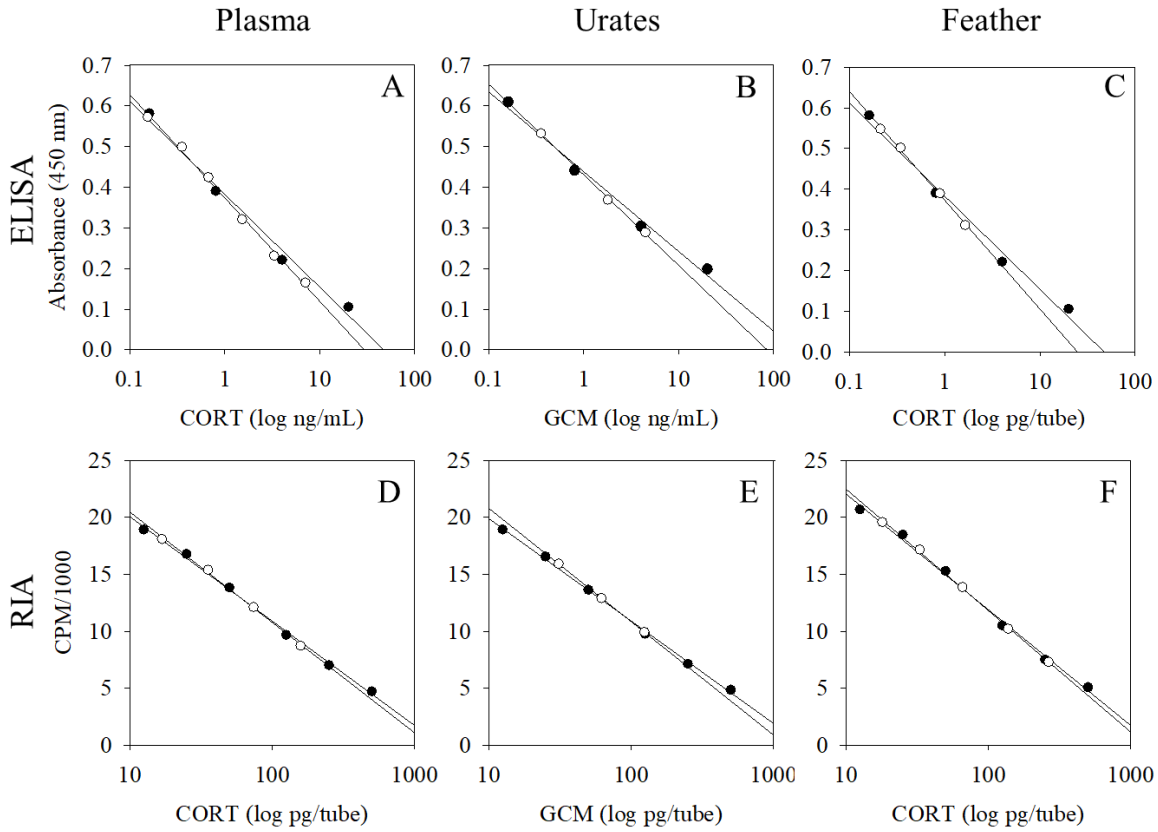
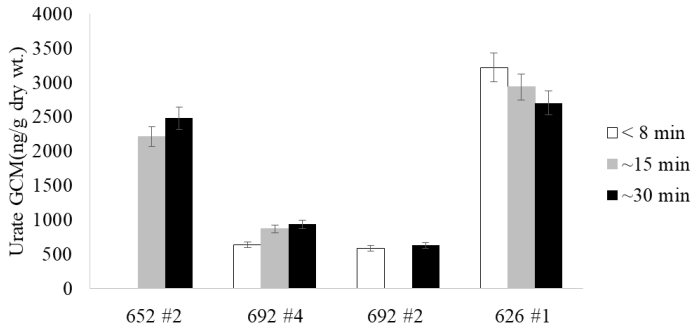


Fig. A1. Radiograph of condor 401 taken 21 June 2010 shows three radio-opaque objects embedded in the wing (birdshot) and one larger radio-opaque object in the bird's digestive tract, later identified as lead buckshot after regurgitation and analysis. Insert panel, lower right: comparison of surgically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

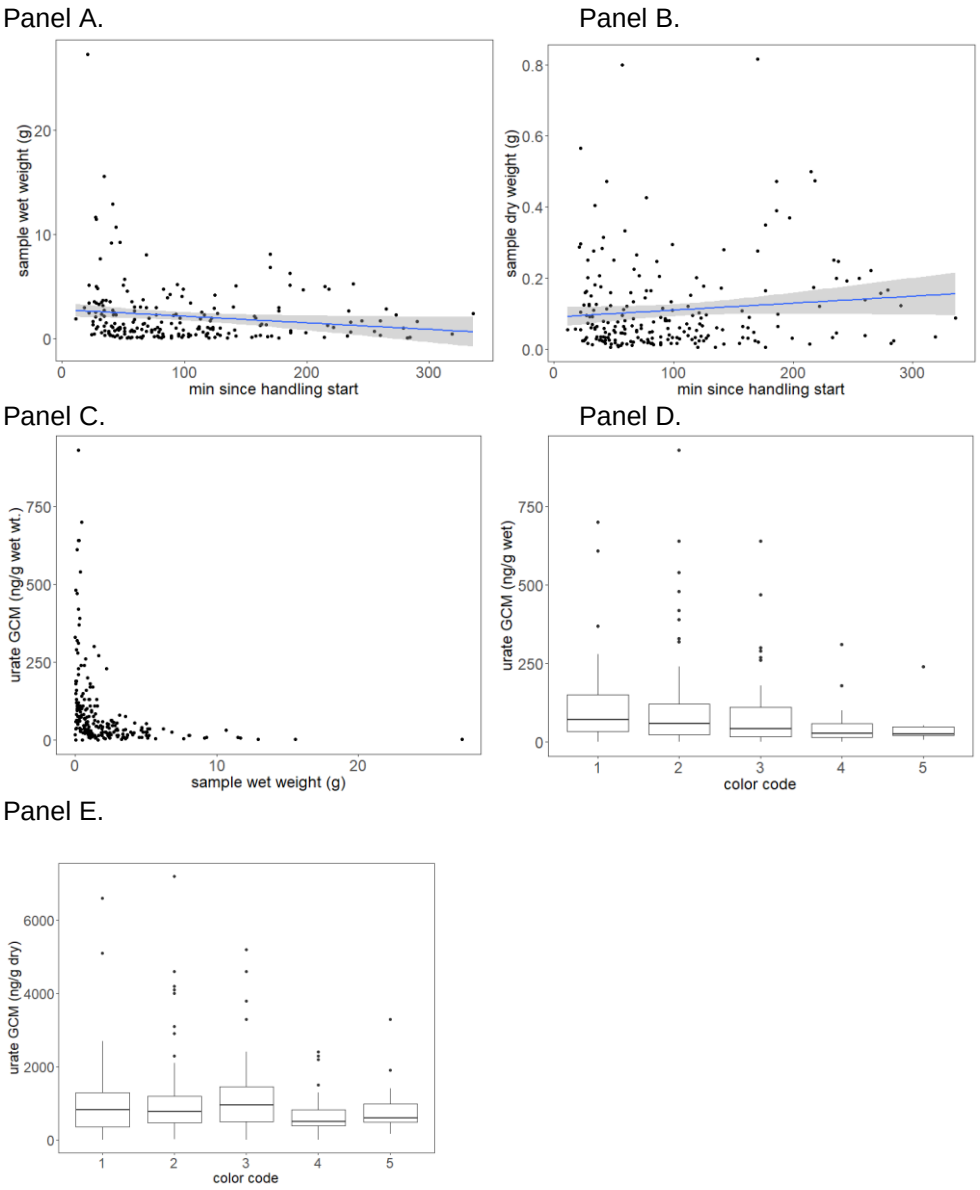
Supplemental Information for Chapter 3



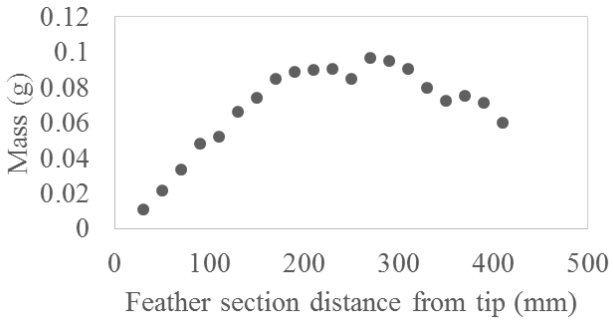
SI Figure 1. Parallelism tests for corticosterone measurement in California condor plasma, urate extract, and feather extract. Corticosterone standards from kit are shown as filled black circles (●) and open circles (○) represent serially diluted samples. (A-C) Standards and samples run on ELISA kit. (D-F) Standards and samples run on RIA kit. Sample type (plasma, urate extract, or feather extract) is indicated by header above each column of plots (Panels A and D show serially diluted plasma, B and E show serially diluted urate extract, C and F show serially diluted feather extract).



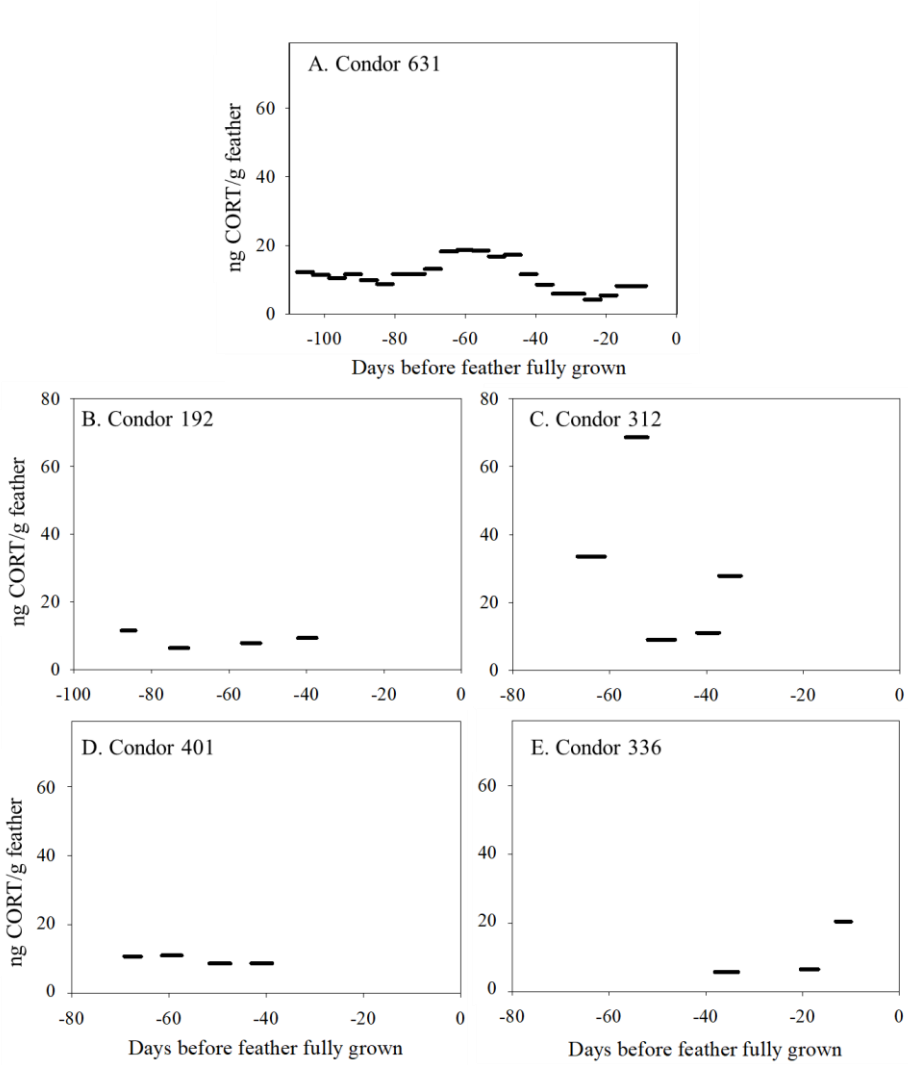
SI Figure 2. Urate GCM concentrations appear stable up to 30 minutes. Four urate samples from three California condors (two samples from one individual) were homogenized via shaking in the field and aliquoted into 2-3 vials. One vial was immediately placed on dry ice after collection (< 8 min since defecation), whereas the remaining vials were placed on dry ice at ~15 and ~30 minutes after collection. Error bars show 6.2% RSD (intra-assay precision for urates by ELISA) and illustrate no measureable change in urate GCM concentration within 30 minutes, except for in the 692 #4 where a small measurable difference was detected between ASAP vs. 15 min to freezing (within 6% RSD).



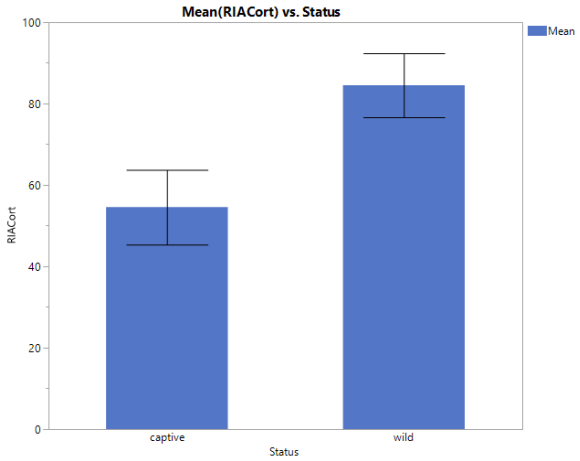
SI Figure 3. Rationale for using dry weight concentrations for urate GCM: (A) We observed a decrease in wet weight (g) of sample over time since handling (Spearman's ρ = -0.19, p= 0.006, n= 216), (B) but not for sample dry weights (g) (Spearman's ρ = 0.02, p = 0.75, n= 216). (C) Wet weight GCM concentration was also negatively correlated with sample wet weight (Spearman's ρ = 0.02, p = 0.75, n= 216). (d) Urate samples of different colors (coded 1-5, ranging from 1=white/clear, 3=yellow, 5=green) had significantly different wet weight GCM concentrations (p=0.03, n=216, one-way ANOVA), an indicator of hydration and potential fecal contamination, whereas dry weight urate GCM were not significantly affected by this variable (p=0.11, n=216, one-way ANOVA). Taken together, this evidence suggests that wet wt. GCM concentrations in urates are more sensitive to hydration states of the individual than dry wt. GCM concentrations. We therefor use ng/g dry wt. for GCM concentrations for our condor urate results.



SI Figure 4. Mass of 2 cm feather vane sections vary along the length of a condor primary feather. Primary feathers have a tapered shape that causes the amount of feather grown for a given time period to vary over feather growth (Bortolotti et al. 2009).

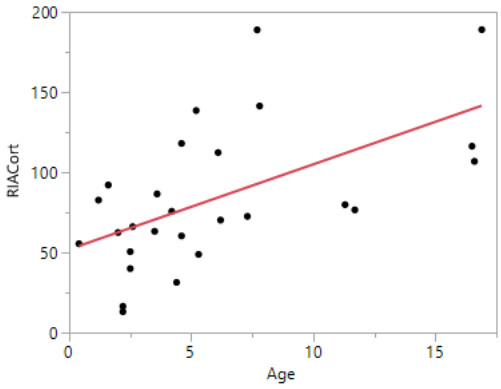


SI Figure 5. Feather CORT concentrations per gram of feather show similar results to CORT concentrations normalized to feather section length (Figure 6 main text, see also SI Table 2).

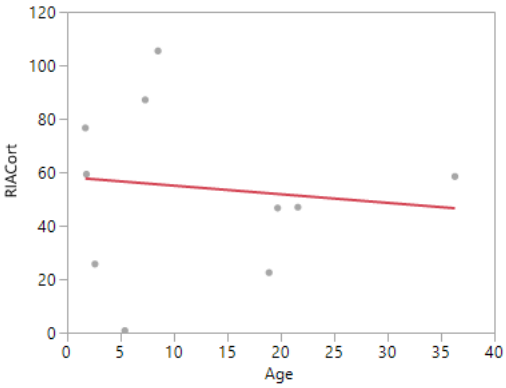


SI Figure 6. Plasma GC (RIACort) values measured in captive (55 ± 31 ng/mL, n=11 samples) vs. wild (85 ± 43 ng/mL, n=30 samples) condors are significantly different(p=0.02, two-tailed t test).

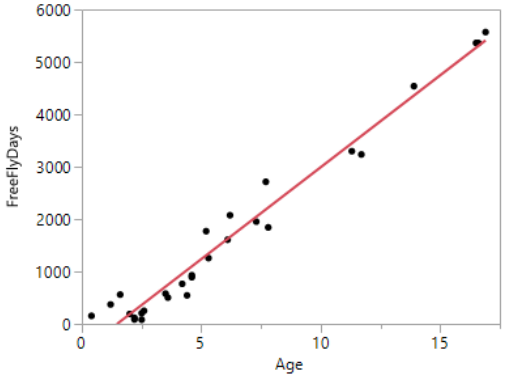
Panel A.



Panel B.



Panel C.



SI Figure 7. (A)Age is correlated with plasma CORT (RIACort) in wild condors (Spearman's ρ=0.48, p=0.01, n=27). (B) Age is not correlated with Plasma CORT (RIACort) in captive condors (Spearman's ρ=-0.25, p=0.45, n=11). (C) For wild condors, age may be a covariate for other influential variables that increases with time in wild (FreeFlyDays) (Spearman's ρ=0.94, p<.001, n=27).

SI Table 1. California condors sampled

Condor ID	Sex [a]	Age [b]	Hatch origin [c]	Status at sampling [d]	Location [e]	Trap Date [f]	Sample collection date	Weight at capture (lbs) [g]	Keel rating [h]	Hydration status [i]
23	M	36	wild	captive	LAZ	NA	6/14/2016	17.7	NA	NA
120	M	21	captive	captive	LAZ	NA	6/14/2016	18.6	NA	NA
159	F	19	captive	captive	LAZ	NA	6/14/2016	18.3	NA	NA
174	F	18	captive	captive	SBZ	NA	7/28/2016	NA	NA	NA
192	F	18	captive	wild	LAZ	NA	6/26/2010	NA	NA	NA
199	M	18	captive	wild	VWS	6/2/2015	6/3/2015	NA	1	0
204	M	12	captive	wild	VWS	6/2/2015	6/3/2015	NA	1	0
209	M	16	captive	wild	VWS	10/28/2015	10/29/2015	22	1	0
236	F	15	captive	wild	PNP	10/8/2015	10/14/2015	17.5	1	0
312	F	6	captive	wild	PNP	NA	5/27/2009	NA	NA	NA
336	F	4	captive	wild	LAZ	NA	9/7/2008	NA	NA	NA
340	M	11	captive	wild	PNP	10/12/2015	10/14/2015	17.8	1	0
351	M	11	captive	wild	PNP	6/9/2015	6/10/2015	17.8	0	0
401	M	3	captive	wild	PNP	NA	5/27/2009	NA	NA	NA
411	M	8	captive	wild	PNP	10/26/2014	10/29/2014	19	1	0
448	M	7	captive	wild	PNP	6/15/2014	6/16/2014	17.9	1	1
463	M	7	captive	wild	PNP	10/26/2015	10/28/2015	19	1	0
464	F	8	captive	captive	SBZ	7/26/2016	7/28/2016	NA	NA	NA
470	M	7	wild	wild	VWS	10/28/2015	10/29/2015	NA	1	0
477	M	7	wild	wild	VWS	5/27/2015	5/28/2015	NA	0	NA
538	F	6	wild	wild	PNP	5/23/2015	5/27/2015	16.4	0	1
544	F	7	captive	captive	SBZ	NA	7/28/2016	NA	NA	NA
547	F	5	captive	wild	VWS	6/2/2015	6/3/2015	NA	1	0
564	M	5	captive	wild	VWS	5/19/2015	6/3/2015	NA	0	1

Condor ID	Sex [a]	Age [b]	Hatch origin [c]	Status at sampling [d]	Location [e]	Trap Date [f]	Sample collection date	Weight at capture (lbs) [g]	Keel rating [h]	Hydration status [i]
567	M	5	wild	wild	VWS	5/19/2015	5/28/2015	NA	1	1
583	F	4	captive	wild	PNP	5/5/2015	5/6/2015	16.5	0	0
597	F	4	captive	wild	PNP	10/2/2015	10/7/2015	19.5	1	1
603	F	5	wild	captive	SBZ	NA	7/28/2016	NA	NA	NA
606	M	4	captive	wild	VWS	10/18/2015	10/21/2015	18.4	1	0
615	M	3	captive	wild	PNP	5/30/2014	6/4/2014	19.6	0	1
626	F	3	captive	wild	PNP	10/27/2014	10/29/2014	19.8	1	0
631	M	2	captive	captive*	LAZ	NA	1/7/2014	NA	1	NA
631	M	4	captive	wild	PNP	10/8/2015	10/14/2015	20	1	1
631	M	4	captive	wild	PNP	NA	11/12/2015	NA	NA	NA
646	F	1	wild	captive*	LAZ	NA	1/7/2014	NA	NA	NA
650	M	1	captive	captive*	LAZ	NA	1/7/2014	NA	NA	NA
650	M	3	captive	wild	PNP	10/8/2015	10/14/2015	18.3	1	0
652	M	1	captive	captive*	LAZ	NA	1/7/2014	NA	NA	NA
652	M	2	captive	wild	VWS	10/22/2014	10/23/2014	NA	1	2
684	F	2	captive	wild	PNP	6/21/2015	6/23/2015	20.6	1	0
684	F	2	captive	wild	PNP	10/12/2015	10/14/2015	18	1	1
687	F	2	captive	wild	PNP	10/5/2015	10/7/2015	20	1	1
688	M	2	captive	wild	VWS	6/2/2015	6/3/2015	20.6	1	0
692	M	2	captive	wild	PNP	6/9/2015	6/10/2015	NA	1	1
700	M	2	captive	wild	PNP	10/2/2015	10/7/2015	18.6	1	1
704	M	2	captive	wild	PNP	10/20/2015	10/21/2015	21.3	1	1
729	M	1	wild	wild	PNP	10/9/2015	10/14/2015	20.6	1	0
745	M	1	wild	wild	VWS	5/27/2015	5/28/2015	NA	1	1
769	F	1	wild	wild	VWS	6/2/2015	6/3/2015	19.7	1	0

a. F = female, M = male
b. Age in years
c. captive = chick hatched and fledged in captivity; wild = chick hatched and fledged in the wild
d. Condor's status at time of sample collection: wild = free-flying in the wild population; captive = long-term captive in zoo facility; captive* = in the captive population during sample collection but slated for release to the wild population
e. Sample collection locations; LAZ = Los Angeles Zoo and Botanical Park, CA; VWS = Ventana Wildlife Society trapping site in Big Sur, CA; PNP = trapping site in Pinnacles National Park, CA
f. Trap date for wild condors. Not applicable (NA) for captive condors which live in flight pens continuously before handling events.
g. Weight at sample collection, not available for all condors.
h. Keel rating is an indicator of body condition measured by palpating keel and pectoral muscle. Condors are scored 1-5 where 1 = emaciated, severely atrophied pectoral muscles in relationship to keel bone, 2 = keel protrudes slightly beyond pectoral muscles, 3 = average, pectoral muscles approximately even with keel bone, 4 = pectoral muscles robust and extend beyond keel bone, 5 = obese, pectoral muscles unusually robust and extend well beyond keel bone. Not scored in all captive birds, and not provided for feather collections. To minimize technician-related bias, we coded these categorical observations as binary (Keel status: 0= breast concave to keel, 1= breast muscle even or convex to keel.
i. Hydration status: 0= dehydrated, 1=well hydrated based on leg skin elasticity after pinching.

SI Table 2. Details for California condor feather sections.

Condor ID	Date Coll.	Section #	Feather length from skin[a](mm)	Mass (g)	Total CORT (ng)	CORT (ng/g)	Section length (cm)[b]	Days of feather growth[c]	pg CORT/ mm feather	Section start[d] (days before full grown)	Section extent[d] (days before full grown)
192	6/26/2010	1 proximal	51	-	-	-	3.4	7.7	-	-11.6	-19.27
192	6/26/2010	2	85	0.0286	-	-	2	4.5	-	-19.27	-23.81
192	6/26/2010	3	105	-	-	-	2.0	4.5	-	-23.81	-28.34
192	6/26/2010	4	125	-	-	-	2.0	4.5	-	-28.34	-32.88
192	6/26/2010	5	145	0.0411	-	-	2.0	4.5	-	-32.88	-37.41
192	6/26/2010	6	165	0.0379	0.35	9.3	2.1	4.8	17	-37.41	-42.18
192	6/26/2010	7	186	-	-	-	2.0	4.5	-	-42.18	-46.71
192	6/26/2010	8	206	0.0304	-	-	2.3	5.2	-	-46.71	-51.93
192	6/26/2010	9	229	0.055	0.43	7.8	2.1	4.8	20	-51.93	-56.69
192	6/26/2010	10	250	-	-	-	2.0	4.5	-	-56.69	-61.22
192	6/26/2010	11	270	0.027	-	-	2.1	4.8	-	-61.22	-65.99
192	6/26/2010	12	291	-	-	-	2.0	4.5	-	-65.99	-70.52
192	6/26/2010	13	311	0.045	0.28	6.3	2.1	4.8	14	-70.52	-75.28
192	6/26/2010	14	332	0.0308	-	-	1.9	4.3	-	-75.28	-79.59
192	6/26/2010	15	351	-	-	-	2.0	4.5	-	-79.59	-84.13
192	6/26/2010	16	371	0.0187	0.22	12	1.6	3.6	14	-84.13	-87.76
192	6/26/2010	17+18 distal	387	0.0171	-	-	3.8	8.6	-	-87.76	-96.37
312	5/27/2009	1 + 2 proximal	45	0.0242	-	-	4.0	9.1	-	-10.2	-19.3
312	5/27/2009	3	85	-	-	-	2.0	4.5	-	-19.3	-23.8
312	5/27/2009	4	105	-	-	-	2.0	4.5	-	-23.8	-28.3
312	5/27/2009	5	125	0.017	-	-	2.0	4.5	-	-28.3	-32.9
312	5/27/2009	6	145	0.017	0.47	28	2.0	4.5	24	-32.9	-37.4

Condor ID	Date Coll.	Section #	Feather length from skin[a](mm)	Mass (g)	Total CORT (ng)	CORT (ng/g)	Section length (cm)[b]	Days of feather growth[c]	pg CORT/ mm feather	Section start[d] (days before full grown)	Section extent[d] (days before full grown)
312	5/27/2009	7	165	0.027	0.3	11	2.0	4.5	15	-37.4	-42.0
312	5/27/2009	8	185	0.0183	-	-	2.0	4.5	-	-42.0	-46.5
312	5/27/2009	9	205	0.037	0.33	9.0	2.5	5.7	13	-46.5	-52.2
312	5/27/2009	10	230	0.0364	1.35	69	2.0	4.5	68	-52.2	-56.7
312	5/27/2009	11	250	0.0223	-	-	1.9	4.3	-	-56.7	-61.0
312	5/27/2009	12	269	0.036	1.21	34	2.5	5.7	48	-61.0	-66.7
312	5/27/2009	13	294	-	-	-	1.0	2.3	-	-66.7	-68.9
312	5/27/2009	14	304	0.0173	-	-	1.8	4.1	-	-68.9	-73.0
312	5/27/2009	15	322	-	-	-	2.6	5.9	-	-73.0	-78.9
312	5/27/2009	16	348	-	-	-	1.1	2.5	-	-78.9	-81.4
312	4/12/2009	17 distal	359	0.057	-	-	5.9	13.4	-	-81.4	-94.8
336	9/7/2008	1+2 proximal	0	0.0419	-	-	3.0	6.8	-	0.0	-6.8
336	9/7/2008	3	30	0.0393	-	-	1.4	3.2	-	-6.8	-10.0
336	9/7/2008	4	44	0.0397	0.82	20	1.4	3.2	58	-10.0	-13.2
336	9/7/2008	5	58	0.0537	-	-	1.6	3.6	-	-13.2	-16.8
336	9/7/2008	6	74	0.062	0.42	6.5	1.6	3.6	26	-16.8	-20.4
336	9/7/2008	7	90	0.0585	-	-	1.8	4.1	-	-20.4	-24.5
336	9/7/2008	8	108	0.0481	-	-	1.8	4.1	-	-24.5	-28.6
336	9/7/2008	9	126	0.0476	-	-	2.1	4.8	-	-28.6	-33.3
336	9/7/2008	10	147	0.062	0.36	5.7	2.1	4.8	17	-33.3	-38.1
336	9/7/2008	11	168	0.0482	-	-	1.9	4.3	-	-38.1	-42.4
336	9/7/2008	12	187	0.0466	-	-	2.0	4.5	-	-42.4	-46.9
336	9/7/2008	13 distal	207	0.0647	-	-	4.0	9.1	-	-46.9	-56.0
401	5/27/2009	1+2 proximal	50	-	-	-	3.5	7.9	-	-11.3	-19.3

Condor ID	Date Coll.	Section #	Feather length from skin[a](mm)	Mass (g)	Total CORT (ng)	CORT (ng/g)	Section length (cm)[b]	Days of feather growth[c]	pg CORT/ mm feather	Section start[d] (days before full grown)	Section extent[d] (days before full grown)
401	5/27/2009	3	77	0.0147	-	-	2.0	4.5	-	-17.5	-22.0
401	5/27/2009	4	94	-	-	-	2.0	4.5	-	-21.3	-25.9
401	5/27/2009	5	116	0.0163	-	-	2.0	4.5	-	-26.3	-30.8
401	5/27/2009	6	134	-	-	-	2.0	4.5	-	-30.4	-34.9
401	5/27/2009	7	152	0.0197	-	-	2.0	4.5	-	-34.5	-39.0
401	5/27/2009	8	171	0.0178	0.16	8.8	2.0	4.5	7.9	-38.8	-43.3
401	5/27/2009	9	190	0.0194	-	-	2.0	4.5	-	-43.1	-47.6
401	5/27/2009	10	209	0.0182	0.16	8.7	2.0	4.5	7.8	-47.4	-51.9
401	5/27/2009	11	228	0.017	-	-	2.0	4.5	-	-51.7	-56.2
401	5/27/2009	12	253	0.012	0.13	11	2.0	4.5	6.6	-57.4	-61.9
401	5/27/2009	13	271	0.0283	-	-	2.0	4.5	-	-61.5	-66.0
401	5/27/2009	14	290	0.0231	0.25	11	2.0	4.5	12	-65.8	-70.3
401	5/27/2009	15	305	0.0252	-	-	2.0	4.5	-	-69.2	-73.7
401	5/27/2009	16	324	0.023	-	-	2.0	4.5	-	-73.5	-78.0
401	5/27/2009	17+18	342	0.0298	-	-	4.0	9.1	-	-77.6	-86.6
401	5/27/2009	19 distal	380	0.0605	-	-	3.8	8.6	-	-86.2	-94.8
631	11/12/2015	1+2 proximal	38	0.0286	0.23	8.1	3.7	8.4	6.3	-8.7	-17.1
631	11/12/2015	3	75	0.029	0.16	5.4	2.0	4.5	7.8	-17.1	-21.6
631	11/12/2015	4	95	0.0725	0.30	4.2	2.0	4.5	15	-21.6	-26.2
631	11/12/2015	5	115	0.0551	0.32	5.8	2.0	4.5	16	-26.2	-30.7
631	11/12/2015	6	135	0.0741	0.44	5.9	2.0	4.5	22	-30.7	-35.2
631	11/12/2015	7	155	0.0604	0.52	8.6	2.0	4.5	26	-35.2	-39.8
631	11/12/2015	8	175	0.0355	0.41	12	2.0	4.5	21	-39.8	-44.3
631	11/12/2015	9	195	0.0353	0.61	17	2.0	4.5	30	-44.3	-48.8

Condor ID	Date Coll.	Section #	Feather length from skin[a](mm)	Mass (g)	Total CORT (ng)	CORT (ng/g)	Section length (cm)[b]	Days of feather growth[c]	pg CORT/ mm feather	Section start[d] (days before full grown)	Section extent[d] (days before full grown)
631	11/12/2015	10	215	0.0343	0.58	17	2.0	4.5	29	-48.8	-53.4
631	11/12/2015	11	235	0.0381	0.70	18	2.0	4.5	35	-53.4	-57.9
631	11/12/2015	12	255	0.0352	0.65	19	2.0	4.5	33	-57.9	-62.4
631	11/12/2015	13	275	0.0281	0.51	18	2.0	4.5	26	-62.4	-67.0
631	11/12/2015	14	295	0.0374	0.49	13	2.0	4.5	24	-67.0	-71.5
631	11/12/2015	15	315	0.0315	0.37	12	2.0	4.5	18	-71.5	-76.1
631	11/12/2015	16	335	0.0358	0.42	12	2.0	4.5	21	-76.1	-80.6
631	11/12/2015	17	355	0.0399	0.35	8.7	2.0	4.5	17	-80.6	-85.1
631	11/12/2015	18	375	0.0412	0.41	9.9	2.0	4.5	20	-85.1	-89.7
631	11/12/2015	19	395	0.0359	0.41	12	2.0	4.5	21	-89.7	-94.2
631	11/12/2015	20	415	0.0296	0.31	10	2.0	4.5	16	-94.2	-98.7
631	11/12/2015	21	435	0.0258	0.29	11	2.0	4.5	15	-98.7	-103.3
631	11/12/2015	22 distal	455	0.0268	0.33	12	2.0	4.5	16	-103.3	-107.8

a. Distance from start of feather section to skin (incorporates exposed calamus length)
b. Section length along rachis axis of feather
c. Days of feather growth/section. Calculated based on feather section length using 0.0441 cm/day growth rate for California condor primary feathers (Finkelstein et al., 2010)
d. These two time points bracket the predicted duration of feather growth (days for which the feather material in this section was perfused during formation in follicle). Based on feather growth calculations from columns A and C.

SI Table 3. CORT extraction recovery for California condor urates. Urate samples were pooled and aliquoted before spiking with corticosterone. All samples were lyophilized and extracted with either 80% MeOH or 95% EtOH and GCM concentration of re-suspended extracts were measured by ELISA. Based on results 80% MeOH was used as urate extraction method.

Extraction Solvent	Mean endogenous GCM concentration (ng/g)[a]	sd	Mean endogenous total GCM in aliquot (ng)[b]	CORT spike (ng)	% spike recovery[c]	sd
80% methanol	19	7.8	5.0	9.2	98	22
95% ethanol	14	2.6	3.6	9.2	43	1.5

a. Unspiked aliquots were extracted by each solvent and their GCM concentration averaged to calculate spike recovery in spiked samples (80% MeOH: n=4 unspiked samples, 95% EtOH: n=3 samples).
b. Included to provide insight into endogenous: spiked hormone ratio as run on ELISA.
c. % spike recovery calculated by first subtracting total endogenous GCM from total GCM plus CORT measured in spiked aliquots of pooled urates, then comparing the difference to the known weight of hormone in added spike (ng) (80% MeOH: n=4 spiked aliquots, 95% EtOH: n=3 spiked aliquots). Total ng endogenous GCM was calculated for spiked samples by multiplying mean endogenous GCM concentration of unspiked samples by aliquot wet weight (g).

SI Table 4. Analytical corticosterone (CORT) spike recovery data

Sample ID	Condor ID if applicable	Sample Type	Assay	Dilution factor (dry)[a]	Dilution factor (wet)[b]	CORT or GCM as run[c]	ng CORT or GCM as run	CORT spike (ng)[d]	% CORT spike recovery
Const_P	pooled	plasma	ELISA	NA	1.5	1.01	0.10	NA	NA
Const_P	pooled	plasma	ELISA	NA	1.5	0.93	0.09	NA	NA
Const_P	pooled	plasma	ELISA	NA	1.5	0.86	0.09	NA	NA
Const_P_sp	pooled	plasma	ELISA	NA	1.5	4.89	0.49	0.46	86
Const_P_sp	pooled	plasma	ELISA	NA	1.5	5.06	0.51	0.46	90
Const_P_sp	pooled	plasma	ELISA	NA	1.5	4.85	0.49	0.46	85
F_unsp	pooled	feather	ELISA	4.7	NA	0.58	0.06	NA	NA
F_unsp	pooled	feather	ELISA	4.7	NA	0.51	0.05	NA	NA
F_unsp	pooled	feather	ELISA	4.7	NA	0.62	0.06	NA	NA
F_sp	pooled	feather	ELISA	4.7	NA	2.42	0.24	0.13	139
F_sp	pooled	feather	ELISA	4.7	NA	2.24	0.22	0.13	125
F_sp	pooled	feather	ELISA	4.7	NA	1.82	0.18	0.13	94
650 #4d	650	urates	ELISA	0.2	2.0	0.83	0.08	NA	NA
650 #4ds	650	urates	ELISA	0.2	2.0	6.44	0.64	0.28	198
652 #4d	652	urates	ELISA	0.2	1.8	1.24	0.12	NA	NA
652 #4ds	652	urates	ELISA	0.2	1.8	6.44	0.64	0.28	183
448 #7ds	448	urates	ELISA	0.1	1.5	0.35	0.04	NA	NA
448 #7ds	448	urates	ELISA	0.1	1.5	3.35	0.33	0.28	106
UP1_19	pooled	urates	ELISA	3.1	37.7	1.11	0.11	NA	NA
UP1_20	pooled	urates	ELISA	3.2	36.4	0.64	0.06	NA	NA
UP1_21	pooled	urates	ELISA	3.1	37.1	0.78	0.08	NA	NA
UP1_22	pooled	urates	ELISA	3.2	38.8	0.73	0.07	NA	NA
UP1_23	pooled	urates	ELISA	1.4	18.2	0.84	0.08	0.04	73
UP1_24	pooled	urates	ELISA	1.4	16.7	0.77	0.08	0.04	67
UP1_25	pooled	urates	ELISA	1.5	16.1	0.80	0.08	0.04	73

Sample ID	Condor ID if applicable	Sample Type	Assay	Dilution factor (dry)[a]	Dilution factor (wet)[b]	CORT or GCM as run[c]	ng CORT or GCM as run	CORT spike (ng)[d]	% CORT spike recovery
UP1_26	pooled	urates	ELISA	1.7	17.7	1.10	0.11	0.04	117
UP1_27	pooled	urates	ELISA	1.3	16.8	1.01	0.10	0.04	105
Feath336pooled_1	336	feather	RIA	33.0	NA	30.96	0.03	NA	NA
Feath336pooled_2	336	feather	RIA	33.0	NA	32.18	0.03	NA	NA
Feath336pooled_3	336	feather	RIA	33.0	NA	33.43	0.03	NA	NA
Feath336pooled_1S	336	feather	RIA	33.0	NA	65.96	0.07	0.03	101
Feath336pooled_2S	336	feather	RIA	33.0	NA	64.11	0.06	0.03	96
Feath336pooled_3S	336	feather	RIA	33.0	NA	67.28	0.07	0.03	105
pooledP(stock)_1	pooled	plasma	RIA	NA	1.0	62.07	0.06	NA	NA
pooledP(stock)_2	pooled	plasma	RIA	NA	1.0	65.27	0.07	NA	NA
pooledP(stock)_3	pooled	plasma	RIA	NA	1.0	65.93	0.07	NA	NA
pooledP(stock)_1S	pooled	plasma	RIA	NA	1.0	117.50	0.12	0.05	99
pooledP(stock)_2S	pooled	plasma	RIA	NA	1.0	113.83	0.11	0.05	92
pooledP(stock)_3S	pooled	plasma	RIA	NA	1.0	114.52	0.11	0.05	93
650 #4	650	urates	RIA	0.1	0.5	25.24	0.03	NA	NA
650 #4b	650	urates	RIA	0.0	0.3	14.26	0.01	NA	NA
650 #4c	650	urates	RIA	0.0	0.1	7.54	0.01	NA	NA
650 #4s	650	urates	RIA	0.1	0.5	76.46	0.08	0.05	102
650 #4bs	650	urates	RIA	0.0	0.3	36.88	0.04	0.03	90
650 #4cs	650	urates	RIA	0.0	0.1	18.48	0.02	0.01	88
448 #3_1	448	urates	RIA	0.0	2.2	88.01	0.09	NA	NA
448 #3_2	448	urates	RIA	0.0	2.2	86.95	0.09	NA	NA
448 #3_3	448	urates	RIA	0.0	2.2	88.74	0.09	NA	NA
448 #3_1S	448	urates	RIA	0.0	2.2	177.91	0.18	0.08	108
448 #3_2S	448	urates	RIA	0.0	2.2	178.08	0.18	0.08	108
448 #3_3S	448	urates	RIA	0.0	2.2	179.20	0.18	0.08	110

a. For feather and urates: mg sample dry/100mL assay buffer
b. For urates: mg sample wet/100mL assay buffer; for plasma: µL sample /100mL assay buffer
c. Plasma CORT, feather CORT, or urate GCM concentration as run (pg/tube for RIA, ng/mL assay buffer for ELISA)
d. Exogenous corticosterone spike in ng as run.

SI Table 5. Samples used in RIA vs. ELISA method comparison

Condor ID	# in series	Sample Type	Wet Mass (g)	Dry Mass (g)	RIA Dilution factor (dry)[a]	RIA Dilution factor (wet)[b]	RIA CORT or GCM as run[c]	RIA CORT or GCM wet sample[d]	RIA CORT or GCM dry sample[e]	RIA Total CORT or GCM (ng)[f]	ELISA Dilution factor (dry)[a]	ELISA Dilution factor (wet)[b]	ELISA CORT or GCM as run[c]	ELISA CORT or GCM wet sample[d]	ELISA CORT or GCM dry sample[e]	ELISA Total CORT or GCM (ng)[f]
312	312_6	Feather	NA	0.017	1.86	NA	0.5	NA	28	0.47	1.12	NA	0.2	NA	20	0.33
312	312_7	Feather	NA	0.027	4.50	NA	0.3	NA	11	0.30	1.80	NA	0.2	NA	13	0.35
312	312_9	Feather	NA	0.037	4.15	NA	0.4	NA	9	0.34	2.49	NA	0.2	NA	7	0.27
312	312_12	Feather	NA	0.036	4.04	NA	0.5	NA	34	1.22	2.42	NA	0.2	NA	9	0.33
336	336_4	Feather	NA	0.040	4.42	NA	0.9	NA	20	0.81	2.65	NA	0.4	NA	14	0.57
336	336_6	Feather	NA	0.062	6.89	NA	0.4	NA	7	0.40	4.14	NA	0.2	NA	6	0.36
336	336_10	Feather	NA	0.064	7.09	NA	0.4	NA	6	0.37	4.25	NA	0.3	NA	7	0.45
606	NA	Plasma	NA	NA	NA	1.0	0.4	42	NA	NA	NA	2.5	1.0	39	NA	NA
615	NA	Plasma	NA	NA	NA	1.0	0.5	49	NA	NA	NA	2.5	0.8	33	NA	NA
626	NA	Plasma	NA	NA	NA	1.0	0.6	63	NA	NA	NA	2.5	0.9	38	NA	NA
631	NA	Plasma	NA	NA	NA	1.0	0.3	26	NA	NA	NA	2.5	0.6	24	NA	NA
646	NA	Plasma	NA	NA	NA	1.0	0.6	59	NA	NA	NA	2.5	1.1	45	NA	NA
650	NA	Plasma	NA	NA	NA	0.5	0.4	71	NA	NA	NA	2.5	1.8	71	NA	NA
652	NA	Plasma	NA	NA	NA	0.5	0.4	77	NA	NA	NA	2.5	1.6	62	NA	NA
631	NA	Plasma	NA	NA	NA	1.0	0.3	26	NA	NA	NA	2.5	0.6	24	NA	NA
646	NA	Plasma	NA	NA	NA	1.0	0.6	59	NA	NA	NA	2.5	1.1	45	NA	NA
650	NA	Plasma	NA	NA	NA	0.5	0.4	71	NA	NA	NA	2.5	1.8	71	NA	NA
652	NA	Plasma	NA	NA	NA	0.5	0.4	77	NA	NA	NA	2.5	1.6	62	NA	NA
231	#1	Urates	3.303	0.164	0.16	3.3	2.1	62	1248	205	0.41	8.3	2.6	32	640	104.98
231	#7	Urates	0.985	0.105	0.16	1.5	4.9	316	2946	311	0.40	3.7	1.9	51	474	50.05
448	#1	Urates	2.554	0.126	0.17	3.4	0.9	25	512	65	0.42	8.5	1.4	16	327	41.30
448	#2	Urates	1.413	0.225	0.03	0.2	0.2	114	720	162	0.40	2.5	0.9	38	238	53.40

Condor ID	# in series	Sample Type	Wet Mass (g)	Dry Mass (g)	RIA Dilution factor (dry)[a]	RIA Dilution factor (wet)[b]	RIA CORT or GCM as run[c]	RIA CORT or GCM wet sample[d]	RIA CORT or GCM dry sample[e]	RIA Total CORT or GCM (ng)[f]	ELISA Dilution factor (dry)[a]	ELISA Dilution factor (wet)[b]	ELISA CORT or GCM as run[c]	ELISA CORT or GCM wet sample[d]	ELISA CORT or GCM dry sample[e]	ELISA Total CORT or GCM (ng)[f]
448	#5	Urates	2.405	0.096	0.08	2.0	0.8	38	946	91	0.21	5.2	0.6	12	290	27.75
448	#6	Urates	1.328	0.047	0.05	1.3	1.1	76	2165	101	0.12	3.3	0.8	23	646	30.15
448	#7	Urates	2.949	0.165	0.04	0.7	0.4	60	1070	176	0.09	1.5	0.4	23	414	68.08
448	1B	Urates	2.250	0.087	0.15	3.8	0.8	19	501	44	0.37	9.6	1.2	12	322	28.09
615	#8	Urates	0.112	0.012	0.40	4	1.7	47	433	5	0.05	0.4	0.2	36	331	4.01
626	#1	Urates	1.906	0.054	0.56	20	2.9	14	2270	27	0.27	9.5	0.7	7	245	13.13
626	#10	Urates	1.698	0.102	0.04	0.6	0.4	69	1153	117	0.10	1.6	1.1	69	1159	117.76
631	#9	Urates	0.885	0.030	0.06	1.8	0.6	30	875	26	0.02	0.4	0.7	16	470	14.18
646	n/a	Urates	0.096	0.059	0.12	0.2	0.3	171	280	16	0.29	0.5	0.3	53	86	5.04
650	#1	Urates	0.228	0.045	1.87	10	1.5	16	300	4	0.22	1.1	0.1	10	52	2.32
650	#4	Urates	6.694	0.680	0.05	0.5	0.3	48	470	320	0.20	2.0	0.8	42	412	280.15
652	#4	Urates	2.513	0.276	0.05	0.5	1.2	246	2242	619	0.20	1.8	1.2	69	626	172.92
626	#10	Urates	1.698	0.102	0.04	0.6	0.4	69	1153	117	0.10	1.6	1.1	69	1159	117.76
631	#9	Urates	0.885	0.030	0.06	1.8	0.6	30	875	26	0.02	0.4	0.7	16	470	14.18
646	n/a	Urates	0.096	0.059	0.12	0.2	0.3	171	280	16	0.29	0.5	0.3	53	86	5.04
650	#1	Urates	0.228	0.045	1.87	10	1.5	16	300	4	0.22	1.1	0.1	10	52	2.32
650	#4	Urates	6.694	0.680	0.05	0.5	0.3	48	470	320	0.20	2.0	0.8	42	412	280.15
652	#4	Urates	2.513	0.276	0.05	0.5	1.2	246	2242	619	0.20	1.8	1.2	69	626	172.92

a. For feather and urates: mg sample dry/100mL assay buffer
b. For urates: mg sample wet/100mL assay buffer; for plasma: µL sample /100mL assay buffer
c. Plasma CORT, feather CORT, or urate GCM concentration as run (ng hormone/mL assay buffer)
d. Plasma CORT or urate GCM concentration in wet sample (ng hormone/mL plasma or ng hormone/g urates wet wt.)
e. Feather CORT, or urate GCM concentration in dry sample (ng hormone/g dry wt. for urates and feather)
f. Total ng CORT or GCM in sample

SI Table 6. Collection and CORT data for plasma samples

Condor ID	Date Coll.	Time since trapped from wild[a] (hr)	Time since initial pen entry[b] (hr)	Time since handling[c] (min)	CORT (ng/mL)
23	6/14/2016	n/a	155	4	58
120	6/14/2016	n/a	n/a	n/a	47
159	6/14/2016	n/a	135	9	47
174	7/28/2016	n/a	10	8	22
199	6/3/2015	23	200	5	107
204	6/3/2015	28	43	6	116
209	10/29/2015	27	65	6	189
340	10/14/2015	43	34	4	77
351	6/10/2015	19	45	4	80
448	6/16/2014	20	14	3	73
463	10/28/2015	46	50	5	141
464	7/28/2016	n/a	109	9	105
470	10/29/2015	21	31	6	189
477	5/28/2015	27	58	7	68
538	5/27/2015	92	14	9	70
544	7/28/2016	n/a	73	8	87
547	6/3/2015	n/a	97	4	112
564	6/3/2015	n/a	128	9	49
567	5/28/2015	223	177	11	139
583	5/6/2015	n/a	42	13	131
597	10/7/2015	118	80	5	118
603	7/28/2016	n/a	141	9	1
606	10/21/2015	72	60	18	60
615	6/4/2014	118	30	9	49
626	10/29/2014	48	20	6	63
631	1/7/2014	n/a	61	10	26
631	10/14/2015	141	12	5	31
646	1/7/2014	n/a	81	10	59
650	1/7/2014	n/a	26	10	71
650	10/14/2015	144	174	5	87
652	1/7/2014	n/a	41	4	77
663	5/28/2015	30	127	6	82
684	10/14/2015	47	137	6	66
687	10/7/2015	45	43	7	51
688	6/3/2015	n/a	155	4	13
692	6/10/2015	19	13	5	16
700	10/7/2015	119	18	5	40

Condor ID	Date Coll.	Time since trapped from wild[a] (hr)	Time since initial pen entry[b] (hr)	Time since handling[c] (min)	CORT (ng/mL)
704	10/21/2015	23	132	7	62
729	10/14/2015	120	73	5	92
745	5/28/2015	n/a	33	13	83
769	6/3/2015	26	74	6	55

a. Time of sample collection as hours since bird was trapped from the wild. Condors are caught and moved into flight pen using a double door trap operated from a blind, and therefor do not see a human until the flight pen entry by technicians on handling days.
b. Time of sample collection as minutes since initial flight pen entry by technicians. This precedes handling start.
c. Time of sample collection as minutes since handling start. Handling start was recorded when condor was trapped in hoop net.

SI Table 7. Collection and GCM data for urate samples

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
23	1	6/14/2016	5.141	0.297	36	620	190	NA	173	22
23	2	6/14/2016	0.396	0.025	31	480	12	NA	190	39
23	3	6/14/2016	0.116	0.016	83	620	9.6	NA	208	57
23	4	6/14/2016	0.224	0.020	110	1200	24	NA	217	66
23	5	6/14/2016	0.147	0.013	110	1200	16	NA	234	83
23	6	6/14/2016	0.241	0.033	120	860	29	NA	269	118
23	7	6/14/2016	0.464	0.061	140	1100	67	NA	287	136
23	8	6/14/2016	0.114	0.017	120	770	13	NA	306	155
23	9	6/14/2016	0.568	0.064	150	1400	88	NA	338	187
23	10	6/14/2016	0.109	0.014	130	1000	14	NA	365	214
23	12	6/14/2016	1.568	0.199	110	860	170	NA	387	236
120	1	6/14/2016	11.485	0.201	7.7	440	89	NA	96	28
120	2	6/14/2016	1.484	0.035	8.1	340	12	NA	104	36
120	3	6/14/2016	2.647	0.332	59	470	160	NA	127	59
120	4	6/14/2016	1.923	0.206	37	340	70	NA	139	71
120	5	6/14/2016	3.928	0.246	25	400	98	NA	154	86
120	6	6/14/2016	2.196	0.115	25	480	55	NA	159	91
120	7	6/14/2016	1.946	0.081	25	590	48	NA	164	96
120	8	6/14/2016	4.068	0.134	17	510	68	NA	166	98
120	9	6/14/2016	1.916	0.059	22	720	42	NA	181	113
120	10	6/14/2016	4.167	0.178	24	570	100	NA	193	125

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
120	11	6/14/2016	3.010	0.172	31	540	93	NA	208	140
120	12	6/14/2016	2.153	0.108	26	510	55	NA	225	157
120	13	6/14/2016	6.853	0.277	25	620	170	NA	238	170
120	14	6/14/2016	0.027	0.005	18	100	0.5	NA	245	177
120	15	6/14/2016	6.230	0.472	36	470	220	NA	254	186
120	16	6/14/2016	4.688	0.370	32	410	150	NA	265	197
120	17	6/14/2016	5.022	0.500	52	530	260	NA	283	215
120	18	6/14/2016	2.653	0.251	47	500	130	NA	302	234
120	19	6/14/2016	5.270	0.247	36	780	190	NA	306	238
120	20	6/14/2016	1.690	0.138	16	200	27	NA	328	260
120	21	6/14/2016	2.263	0.158	43	620	97	NA	341	273
120	22	6/14/2016	1.649	0.122	59	800	97	NA	358	290
120	23	6/14/2016	0.458	0.034	80	1100	37	NA	387	319
120	25	6/14/2016	2.371	0.088	51	1400	120	NA	404	336
159	1	6/14/2016	2.481	0.566	6	26	15	NA	148	22
159	2	6/14/2016	0.713	0.206	32	110	23	NA	164	38
159	4+5	6/14/2016	3.565	0.799	34	150	120	NA	183	57
159	6	6/14/2016	0.798	0.109	34	250	27	NA	225	99
159	7	6/14/2016	0.967	0.121	32	260	31	NA	238	112
159	9	6/14/2016	1.043	0.087	23	280	24	NA	249	123
159	10	6/14/2016	5.025	0.280	16	290	81	NA	268	142
159	12	6/14/2016	1.224	0.060	9.8	200	12	NA	288	162
159	13	6/14/2016	8.079	0.816	16	160	130	NA	296	170
159	14	6/14/2016	5.108	0.389	25	330	130	NA	312	186

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
159	15	6/14/2016	4.742	0.474	36	360	170	NA	344	218
159	17	6/14/2016	0.643	0.045	35	500	22	NA	362	236
159	20	6/14/2016	2.847	0.221	47	610	130	NA	391	265
159	22	6/14/2016	0.107	0.023	64	290	6.8	NA	410	284
174	1	7/28/2016	1.323	0.124	53	560	70	NA	27	25
174	3	7/28/2016	0.402	0.050	100	840	42	NA	84	82
174	4	7/28/2016	2.532	0.151	63	1100	160	NA	116	114
174	5	7/28/2016	0.815	0.054	51	770	42	NA	143	141
174	6	7/28/2016	0.200	0.024	63	530	13	NA	170	168
174	7	7/28/2016	1.278	0.174	170	1200	210	NA	219	217
174	9	7/28/2016	0.982	0.167	130	760	130	NA	281	279
174	11	7/28/2016	0.075	0.016	0.8	4	0.1	NA	284	282
209	1	10/29/2015	3.561	0.175	75	1500	270	28.0	98	39
209	2	10/29/2015	0.823	0.042	100	2000	84	28.1	105	46
209	3	10/29/2015	0.667	0.046	130	1900	86	28.3	115	56
209	4	10/29/2015	0.127	0.022	160	900	20	28.4	123	64
209	5	10/29/2015	0.639	0.067	240	2300	150	28.6	132	73
209	6	10/29/2015	0.045	0.013	190	670	8.7	28.8	148	89
209	7	10/29/2015	0.172	0.016	94	1000	16	29.1	162	103
209	8	10/29/2015	1.153	0.038	95	2900	110	29.2	171	112
340	1	10/14/2015	2.053	0.033	7.5	470	15	43.2	57	27
340	3	10/14/2015	2.321	0.069	16	530	37	43.3	62	32
340	4	10/14/2015	3.682	0.063	14	840	53	43.4	66	36
340	6	10/14/2015	2.473	0.047	24	1300	60	43.5	73	43

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
340	7	10/14/2015	0.069	0.011	60	360	4.1	43.7	86	56
340	8	10/14/2015	0.139	0.015	100	960	14	43.8	91	61
340	9	10/14/2015	0.230	0.028	210	1700	48	44.1	108	78
340	10	10/14/2015	0.044	0.016	180	490	8	44.1	111	81
340	11	10/14/2015	0.265	0.034	310	2400	81	44.3	119	89
340	12	10/14/2015	0.129	0.017	290	2200	38	44.4	128	98
340	13	10/14/2015	0.143	0.024	320	1900	45	44.9	155	125
340	14	10/14/2015	0.422	0.031	240	3300	100	44.9	157	127
340	15	10/14/2015	0.261	0.015	230	4000	61	45.2	172	142
340	16	10/14/2015	0.315	0.030	390	4100	120	45.5	192	162
340	17	10/14/2015	0.223	0.020	420	4600	94	45.6	201	171
351	1	6/10/2015	0.316	0.029	61	670	19	19.1	76	35
351	2	6/10/2015	2.729	0.079	26	880	70	19.2	82	41
351	4	6/10/2015	0.702	0.047	21	310	15	19.3	92	51
448	1	6/16/2014	4.804	0.126	25	510	120	20.2	40	29
448	2	6/16/2014	1.413	0.225	110	720	160	20.8	78	67
448	3	6/16/2014	4.732	0.295	40	3800	190	21.4	110	99
448	4	6/16/2014	0.961	0.071	76	1000	73	21.6	122	111
448	5	6/16/2014	2.405	0.096	38	950	91	21.8	138	127
448	6	6/16/2014	1.328	0.047	76	2200	100	22.5	178	167
448	7	6/16/2014	2.949	0.165	36	640	110	22.7	188	177
463	1	10/28/2015	7.668	0.061	4.6	580	35	22.2	76	31
463	2	10/28/2015	1.188	0.057	22	470	26	22.2	79	34
463	3	10/28/2015	0.309	0.035	65	560	20	22.3	84	39

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
463	4	10/28/2015	0.260	0.034	64	490	17	22.3	86	41
463	5	10/28/2015	0.189	0.026	75	550	14	22.4	88	43
463	6	10/28/2015	0.272	0.032	96	820	26	22.4	90	45
463	8	10/28/2015	0.131	0.015	110	940	14	22.4	93	48
463	10	10/28/2015	0.820	0.095	160	1400	130	22.6	102	57
464	1	7/28/2016	0.362	0.023	91	1400	33	NA	124	24
464	2	7/28/2016	1.058	0.122	180	1600	190	NA	147	47
464	3	7/28/2016	4.224	0.204	55	1100	230	NA	188	88
464	4	7/28/2016	2.296	0.095	53	1300	120	NA	216	116
464	5	7/28/2016	0.072	0.005	0.8	12	0.1	NA	220	120
464	6	7/28/2016	1.358	0.089	300	4600	410	NA	263	163
464	7	7/28/2016	0.747	0.098	260	2000	190	NA	287	187
464	8	7/28/2016	1.698	0.192	270	2400	460	NA	345	245
470	1	10/29/2015	1.116	0.123	29	260	32	21.4	54	29
470	2	10/29/2015	0.499	0.025	100	2100	51	21.6	63	38
470	3	10/29/2015	0.065	0.005	150	1800	9.6	21.7	72	47
470	4	10/29/2015	0.032	0.010	330	1000	11	21.8	78	53
470	5	10/29/2015	0.074	0.011	480	3100	35	22.0	90	65
470	6	10/29/2015	0.165	0.020	610	5100	100	22.2	100	75
470	7	10/29/2015	0.225	0.028	640	5200	140	22.5	120	95
470	8	10/29/2015	0.385	0.050	540	4200	210	22.7	134	109
470	9	10/29/2015	0.497	0.053	700	6600	350	22.9	144	119
470	10	10/29/2015	0.273	0.038	640	4600	180	23.1	154	129
470	11	10/29/2015	0.252	0.032	930	7200	230	23.6	183	158

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
538	1	5/27/2015	2.286	0.114	42	850	97	92.3	49	44
538	2	5/27/2015	0.959	0.123	18	140	17	92.4	52	47
538	4	5/27/2015	0.108	0.007	38	560	4.1	92.5	59	54
538	5	5/27/2015	0.759	0.046	49	810	37	92.5	62	57
538	7	5/27/2015	1.796	0.044	47	1900	85	92.6	64	59
538	8	5/27/2015	1.180	0.032	90	3300	110	92.7	75	70
544	1	7/28/2016	2.545	0.095	18	470	45	NA	92	27
544	2	7/28/2016	1.549	0.164	130	1200	190	NA	146	81
544	3	7/28/2016	2.615	0.349	63	470	160	NA	242	177
544	4	7/28/2016	1.091	0.120	170	1500	180	NA	287	222
544	7	7/28/2016	0.671	0.200	130	430	87	NA	320	255
547	1	6/3/2015	2.979	0.056	14	720	41	27.8	111	18
547	3	6/3/2015	0.928	0.017	20	1100	18	27.9	121	28
547	4	6/3/2015	1.215	0.024	22	1100	27	28.1	133	40
547	5	6/3/2015	0.770	0.017	31	1400	24	28.2	136	43
547	7	6/3/2015	0.785	0.026	56	1700	44	28.7	166	73
547	8	6/3/2015	2.328	0.081	43	1200	100	29.0	186	93
547	9	6/3/2015	1.514	0.040	44	1600	66	29.1	189	96
547	10	6/3/2015	0.625	0.027	46	1100	29	29.4	208	115
547	11	6/3/2015	0.349	0.017	38	810	13	29.4	211	118
547	12	6/3/2015	0.940	0.023	34	1400	32	29.5	214	121
547	14	6/3/2015	0.976	0.038	42	1100	41	29.6	222	129
583	2	5/6/2015	3.135	0.276	80	910	250	24.0	62	33
583	3	5/6/2015	2.241	0.427	230	1200	520	24.7	106	77

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
583	5	5/6/2015	0.153	0.030	470	2400	71	24.8	111	82
606	1	10/21/2015	3.467	0.092	7.8	300	27	72.7	74	32
606	3	10/21/2015	1.435	0.032	5.9	270	8.5	72.9	85	43
606	4	10/21/2015	0.986	0.026	12	440	11	72.9	88	46
606	5	10/21/2015	0.993	0.019	9.2	480	9.1	73.0	93	51
606	6	10/21/2015	3.703	0.133	18	490	65	73.2	108	66
606	7	10/21/2015	0.701	0.049	68	970	47	73.3	111	69
606	8	10/21/2015	0.198	0.016	51	620	10	73.5	124	82
606	9	10/21/2015	0.418	0.059	74	520	31	73.7	137	95
606	10	10/21/2015	0.303	0.033	75	690	23	73.8	141	99
606	11	10/21/2015	0.315	0.029	100	1100	33	73.9	147	105
606	12	10/21/2015	0.884	0.037	23	550	20	74.2	165	123
606	13	10/21/2015	0.043	0.005	37	360	1.6	74.4	177	135
626	1	10/29/2014	1.906	0.054	14	2300	28	48.1	25	11
626	2	10/29/2014	1.083	0.022	6.8	330	7.4	48.6	55	41
626	3	10/29/2014	1.398	0.052	16	430	22	48.7	64	50
626	4	10/29/2014	0.406	0.025	28	460	12	48.7	65	51
626	5	10/29/2014	0.663	0.056	70	830	47	48.9	73	59
626	6	10/29/2014	0.619	0.058	82	880	51	49.1	87	73
626	7	10/29/2014	1.015	0.085	130	1500	130	49.4	107	93
626	8	10/29/2014	0.400	0.033	110	1300	44	49.6	117	103
626	10	10/29/2014	1.698	0.102	41	690	70	49.9	135	121
626	11	10/29/2014	0.217	0.032	280	1900	62	51.8	247	233
626	13	10/29/2014	0.286	0.039	370	2700	100	52.2	274	260

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
631	1	10/14/2015	27.300	0.288	2.9	270	79	141.2	28	21
631	3	10/14/2015	3.311	0.092	3	110	10	141.4	37	30
631	4	10/14/2015	0.528	0.012	0.023	1	0.012	141.4	39	32
631	5	10/14/2015	15.550	0.404	2.8	110	44	141.5	41	34
631	6	10/14/2015	12.907	0.315	3.2	130	41	141.6	48	41
631	7	10/14/2015	5.686	0.064	5	450	29	141.7	58	51
631	8	10/14/2015	4.526	0.076	8.4	500	38	141.8	60	53
631	9	10/14/2015	2.971	0.052	14	770	40	142	76	69
631	10	10/14/2015	1.048	0.018	12	700	13	142.2	85	78
631	11	10/14/2015	4.708	0.077	9.6	580	45	142.3	90	83
631	12	10/14/2015	5.205	0.080	15	1000	80	142.5	101	94
631	13	10/14/2015	3.399	0.061	19	1100	66	142.6	112	105
631	14	10/14/2015	1.965	0.071	42	1200	83	142.9	129	122
631	15	10/14/2015	2.895	0.066	31	1300	88	143	131	124
631	17	10/14/2015	1.945	0.064	60	1800	120	143.5	165	158
631	18	10/14/2015	0.572	0.031	110	2000	62	144.2	206	199
663	1	5/28/2015	1.851	0.149	24	300	45	30.3	152	31
663	2	5/28/2015	0.732	0.126	97	560	71	30.3	156	35
663	4	5/28/2015	0.886	0.144	200	1200	170	31.0	197	76
663	5	5/28/2015	0.093	0.022	190	800	18	31.1	200	79
684	1	6/23/2015	3.410	0.104	31	1000	100	45.3	105	22
684	2	6/23/2015	0.446	0.027	33	560	15	45.3	109	26
684	3	6/23/2015	2.741	0.181	58	870	160	45.5	117	34
684	4	6/23/2015	0.857	0.074	73	850	63	45.7	128	45

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
687	2	10/7/2015	9.168	0.283	7	230	65	45.7	76	40
687	3	10/7/2015	9.265	0.159	8.3	480	77	45.8	83	47
687	4	10/7/2015	3.054	0.081	14	530	43	46.0	95	59
687	5	10/7/2015	8.020	0.266	15	470	120	46.1	105	69
687	7	10/7/2015	0.590	0.021	24	660	14	46.3	115	79
692	1	6/10/2015	3.544	0.159	4.9	110	17	19.5	34	26
692	2	6/10/2015	4.995	0.250	26	490	130	19.6	36	28
692	3	6/10/2015	2.129	0.061	4.9	170	11	19.7	42	34
692	4	6/10/2015	10.668	0.471	32	530	340	19.8	52	44
692	5	6/10/2015	5.112	0.250	7.1	130	36	19.9	58	50
692	6	6/10/2015	1.556	0.111	45	630	69	20.1	66	58
692	7	6/10/2015	1.018	0.121	20	160	20	20.1	69	61
692	8	6/10/2015	3.033	0.159	10	200	32	20.2	72	64
692	10	6/10/2015	2.973	0.164	55	1000	160	20.4	85	77
700	1	10/7/2015	2.434	0.055	8.1	360	20	119.5	35	22
700	2	10/7/2015	1.638	0.024	0.028	1.9	0.046	119.5	38	25
700	3	10/7/2015	3.391	0.073	7.7	360	26	119.6	41	28
700	4	10/7/2015	3.663	0.109	8	270	29	119.6	46	33
700	6	10/7/2015	0.663	0.064	41	420	27	120.1	71	58
700	7	10/7/2015	0.896	0.066	50	680	45	120.2	78	65
700	8	10/7/2015	1.435	0.089	84	1300	120	120.6	102	89
745	1	5/28/2015	11.665	0.162	7.2	520	84	28.5	47	27
745	2	5/28/2015	2.236	0.058	24	910	53	28.8	62	42
745	4	5/28/2015	1.397	0.085	63	1000	87	29.2	86	66

Condor ID	# in series	Date Collected	Wet mass (g)	Dry mass (g)	GCM (ng/g wet)	GCM (ng/g dry)	Total GCM (ng)	Time since trapped from wild [a] (hr)	Time since initial pen entry[b] (min)	Time since handling[c] (min)
745	5	5/28/2015	0.894	0.047	60	1200	54	29.4	100	80
745	6	5/28/2015	0.201	0.018	53	580	11	29.6	109	89
745	7	5/28/2015	1.089	0.201	110	590	120	30.1	139	119

a. Time of sample collection as hours since bird was trapped from the wild. Condors are caught and moved into flight pen using a double door trap operated from a blind, and therefor do not see a human until the flight pen entry by technicians on handling days.
b. Time of sample collection as minutes since initial flight pen entry by technicians. This precedes handling start.
c. Time of sample collection as minutes since handling start. Handling start was recorded when condor was trapped in hoop net.

SI Table 8. Multiple linear regression model averaged parameter estimates for plasma CORT levels.

Parameter	*N-models*[a]	*Sum wt.*[b]	Estimate[c]	SE[d]	90% CI[e] Upper	90% CI[e] Lower
Intercept	--	--	47.00	15.69	72.73	21.27
Age	27	1.00	72.64	21.40	107.74	37.54
Season	9	0.34	9.83	7.42	22.00	-2.33
Keel	8	0.24	-10.32	10.71	7.24	-27.88
Hydration	7	0.21	7.99	7.50	20.29	-4.30
Min Since Handling	7	0.17	9.31	13.73	31.82	-13.21
Sex	6	0.14	-3.53	9.08	11.36	-18.41
Hr Since Trapped	5	0.12	6.49	15.26	31.51	-18.54
Min Since Entry	4	0.11	7.42	13.39	29.39	-14.54

[a]Number of competitive models (listed in Table 2) including the parameter. [b]Summed Akaike weights for all models with parameter. [c]Weighted average beta coefficient. [d]Model averaged standard error. [e]90% confidence interval for parameter estimate.

SI Table 9. Multiple linear regression model averaged parameter estimates for 1st urate GCM.

Parameter	*N-models*[a]	*Sum wt.*[b]	Estimate[c]	SE[d]	90% CI[e] Upper	90% CI[e] Lower
Intercept	--	--	171	153	422	-80.1
Age	13	0.79	764	285	1230	297
Sex	8	0.66	-155	68.2	-43.5	-267
Plasma CORT	9	0.45	304	157	561	47.5
Season	5	0.27	102	63.6	206	-2.03
Min Since Handling	3	0.11	167	132	384	-50.0
Min Since Entry	2	0.09	217	225	586	-153
Hr Since Trapped	2	0.05	51.2	185	354	-252

[a]Number of competitive models (listed in Table 2) including the parameter. [b]Summed Akaike weights for all models with parameter. [c]Weighted average beta coefficient. [d]Model averaged standard error. [e]90% confidence interval for parameter estimate.

Supplemental Information for Chapter 4

Table S1. Individual California condors and associated life history variables. See Table 2 for variable definitions not in footnotes below.

Condor ID	Date Coll.[a]	Site[b]	Age	Sex	Status[c]	Breeding[d]	Season	CurrentPb	PropHiPb Life	PropHiPb 18	Free Flying Days[e]	Free Flying Days 18[f]	DayAbsent	DaysAbsent 18	Yrs MMFed
23	6/14/2016	LAZ	36.4	M	captive	NA	0	2.5	0.00	0.00	703	0	0	0	0
120	6/14/2016	LAZ	21.6	M	captive	NA	0	2.5	0.00	0.00	0	0	0	0	0
159	6/14/2016	LAZ	19.7	F	captive	NA	0	2.5	0.00	0.00	212	0	0	0	0
168	10/26/2015	VWS	18.9	M	wild	N	0	2.5	0.00	0.00	6051	655	1157	326	7
174	7/28/2016	SBZ	18.9	F	captive	NA	0	2.5	0.00	0.00	0	0	0	0	0
199	6/3/2015	VWS	16.6	M	wild	Y	0	9	0.20	0.00	5363	546	998	301	8
204	6/3/2015	VWS	16.5	M	wild	N	0	8	0.18	0.00	5363	547	998	208	11
209	10/16/2014	VWS	15.8	M	wild	N	0	9.2	0.19	0.50	5195	524	769	242	5
209	10/29/2015	VWS	16.9	M	wild	N	0	22	0.18	0.00	5571	545	935	269	5
219	10/26/2015	VWS	15.9	M	wild	N	0	170	0.43	0.67	5086	519	908	170	7
231	10/16/2014	VWS	14.8	F	wild	N	0	53	0.07	1.00	4852	547	956	310	6
236	10/29/2014	PNP	14.0	F	wild	N	0	43	0.22	0.75	4210	545	700	206	5
236	10/14/2015	PNP	14.0	F	wild	N	0	29	0.26	0.67	4539	525	739	126	5
340	10/14/2015	PNP	11.7	M	wild	N	0	37	0.61	0.50	3236	502	333	64	5
351	6/10/2015	PNP	11.3	M	wild	N	0	15	0.22	0.00	3298	539	556	171	5
411	10/29/2014	PNP	8.7	M	wild	N	0	100	0.54	1.00	2395	521	271	94	1
431	10/16/2014	VWS	7.7	M	wild	N	0	37	0.44	1.00	2034	512	385	185	1
448	6/16/2014	PNP	7.3	M	wild	N	0	92	0.43	1.00	1954	632	344	244	1
463	10/28/2015	PNP	7.8	M	wild	N	0	38	0.50	1.00	1844	377	291	82	3

Condor ID	Date Coll.[a]	Site[b]	Age	Sex	Status[c]	Breeding[d]	Season	CurrentPb	PropHiPb Life	PropHiPb 18	Free Flying Days[e]	Free Flying Days 18[f]	DayAbsent	DaysAbsent 18	Yrs MMFed
464	7/28/2016	SBZ	8.5	F	captive	NA	0	2.5	0.00	0.00	0	0	0	0	0
470	10/29/2015	VWS	7.7	M	wild	N	0	25	0.14	0.00	2714	542	724	233	6
477	5/28/2015	VWS	7.3	M	wild	N	1	44	0.17	0.00	2549	541	646	213	4
534	10/16/2014	VWS	5.5	F	wild	N	0	13	0.00	0.00	1436	543	374	281	3
538	5/27/2015	PNP	6.2	F	wild	N	1	170	0.50	0.67	2076	535	523	219	3
543	10/21/2015	PNP	6.6	F	wild	N	0	24	0.17	0.50	1776	512	583	261	2
544	7/28/2016	SBZ	7.3	F	captive	NA	0	2.5	0.00	0.00	0	0	0	0	0
547	6/3/2015	VWS	6.1	F	wild	N	0	37	0.20	1.00	1609	546	442	215	3
564	5/28/2015	VWS	5.3	M	wild	N	1	44	0.67	0.67	1258	534	339	229	2
567	5/28/2015	VWS	5.2	M	wild	N	1	13	0.40	0.00	1772	534	360	144	3
569	10/9/2014	VWS	4.6	F	wild	N	0	25	0.00	0.00	960	525	217	129	2
583	10/16/2014	VWS	4.5	F	wild	N	0	33	0.50	0.33	954	509	269	187	2
583	5/6/2015	PNP	5.1	F	wild	N	1	12	0.40	0.20	1153	522	341	194	2
597	10/7/2015	PNP	4.6	F	wild	N	0	21	0.25	0.25	894	512	344	230	0
603	7/28/2016	SBZ	5.4	F	captive		0	2.5	0.00	0.00	0	0	0	0	0
606	4/9/2014	PNP	3.0	M	wild	N	1	27	1.00	0.67	384	379	66	72	0
606	5/28/2015	VWS	4.2	M	wild	N	1	52	0.75	0.67	786	527	254	212	2
606	10/21/2015	PNP	4.6	M	wild	N	0	41	0.83	1.00	930	534	338	267	2
615	6/4/2014	PNP	3.2	M	wild	N	0	16	0.00	0.00	432	432	215	211	0
615	5/28/2015	VWS	4.2	M	wild	N	1	69	0.00	0.33	766	517	378	244	0
626	10/29/2014	PNP	3.5	F	wild	N	0	31	0.00	0.00	580	520	145	142	0
626	6/23/2015	PNP	4.2	F	wild	N	0	30	0.00	0.00	808	510	244	194	0
626	10/29/2015	VWS	4.5	F	wild	N	0	98	0.00	0.25	933	508	301	216	1
631	1/7/2014	LAZ	2.6	M	captive	NA	1	2.5	0.00	0.00	0	0	0	0	0
631	6/3/2015	VWS	4.1	M	wild	N	0	2.5	0.00	0.00	383	383	191	191	0

Condor ID	Date Coll.[a]	Site[b]	Age	Sex	Status[c]	Breeding[d]	Season	CurrentPb	PropHiPb Life	PropHiPb 18	Free Flying Days[e]	Free Flying Days 18[f]	DayAbsent	DaysAbsent 18	Yrs MMFed
631	10/14/2015	PNP	4.4	M	wild	N	0	28	0.00	0.00	546	541	228	227	0
646	1/7/2014	LAZ	1.8	F	captive		1	2.5	0.00	0.00	0	0	0	0	0
650	1/7/2014	LAZ	1.8	M	captive	NA	1	2.5	0.00	0.00	0	0	0	0	0
650	2/25/2014	VWS	1.9	M	captive	N	1	2.5	0.00	0.00	0	0	0	0	0
650	10/14/2015	PNP	3.6	M	wild	N	0	17	0.00	0.00	504	504	204	204	2
652	1/7/2014	LAZ	1.7	M	captive	NA	1	2.5	0.00	0.00	0	0	0	0	0
652	10/23/2014	VWS	2.5	M	wild	N	0	2.5	0.00	0.00	174	174	94	94	1
663	5/28/2015	VWS	3.2	M	wild	N	1	34	0.33	0.50	1138	539	442	177	1
663	10/21/2015	PNP	3.7	M	wild	N	0	11	0.25	0.33	1275	532	514	230	1
684	6/23/2015	PNP	2.3	F	wild	N	0	30	0.00	0.00	142	142	12	12	0
684	10/14/2015	PNP	2.6	F	wild	N	0	55	0.00	0.33	252	252	41	41	0
687	10/7/2015	PNP	2.5	F	wild	N	0	28	0.00	0.00	207	207	40	40	0
688	6/3/2015	VWS	2.2	M	wild	N	0	26	0.00	0.00	87	87	26	26	0
692	6/10/2015	PNP	2.2	M	wild	N	0	30	0.00	0.00	117	87	17	26	0
700	10/7/2015	PNP	2.5	M	wild	N	0	48	0.00	0.00	83	83	12	12	0
704	10/21/2015	PNP	2.1	M	wild	N	0	58	0.50	0.75	191	191	89	89	0
729	10/14/2015	PNP	1.6	M	wild	N	0	2.5	0.00	0.00	561	543	464	447	1
745	5/28/2015	VWS	1.2	M	wild	N	1	17	0.00	0.00	373	373	246	246	0
769	6/3/2015	VWS	0.4	M	wild	N	0	14	0.00	0.00	155	155	113	113	0

[a]Date of handling event and sample collection. [b]Site of handling event and sample collection: LAZ = Los Angeles zoo and Botanical Gardens, SBZ = Santa Barbara Zoo, VWS = Ventana Wildlife Society in Big Sur, PNP = Pinnacles National Park. [c]Status: captive = zoo captive condors, wild = free-flying condors. [d]Sum lifetime days free flying in the wild (days captive in zoo for treatment or in flight pens in the field not counted). [e]Sum days free flying in the last 18 months before sample collection and associated captivity. See Table 2 for definitions of remaining column headers.

Table S2. Plasma samples and associated stressor variable values.

Condor ID	DateColl[a]	Site[b]	CORT (ng/mL)	FlightPen (hr)	TechEntry (min)	MinSinceHandl (min)
23	6/14/2016	LAZ	58	NA	151	4
120	6/14/2016	LAZ	47	NA	NA	NA
159	6/14/2016	LAZ	47	NA	126	9
174	7/28/2016	SBZ	22	96	2	8
199	6/3/2015	VWS	107	19	195	5
204	6/3/2015	VWS	116	28	37	6
209	10/29/2015	VWS	189	26	59	6
236	10/29/2014	PNP	63	NA	NA	NA
236	10/14/2015	PNP	32	139	96	5
340	10/14/2015	PNP	77	42	30	4
351	6/10/2015	PNP	80	18	41	4
411	10/29/2014	PNP	44	NA	NA	NA
448	6/16/2014	PNP	73	20	11	3
463	10/28/2015	PNP	141	21	45	5
464	7/28/2016	SBZ	105	NA	100	9
470	10/29/2015	VWS	189	21	25	6
477	5/28/2015	VWS	68	26	51	7
538	5/27/2015	PNP	70	91	5	9
544	7/28/2016	SBZ	87	NA	65	8
547	6/3/2015	VWS	112	26	93	4
564	5/28/2015	VWS	49	218	119	9
567	5/28/2015	VWS	139	220	166	11
583	5/6/2015	PNP	131	23	29	13
597	10/7/2015	PNP	118	116	75	5
603	7/28/2016	SBZ	1	NA	132	9
606	4/9/2014	PNP	42	164	40	8
606	5/28/2015	VWS	61	26	53	6
606	10/21/2015	PNP	60	71	42	18
615	6/4/2014	PNP	49	117	21	9
615	5/28/2015	VWS	76	28	158	8
626	10/29/2014	PNP	63	48	14	6
626	6/23/2015	PNP	43	142	43	6
626	10/29/2015	VWS	30	46	51	3
631	1/7/2014	LAZ	26	NA	51	10
631	6/3/2015	VWS	72	24	116	5
631	10/14/2015	PNP	31	141	7	5
646	1/7/2014	LAZ	59	NA	71	10
650	1/7/2014	LAZ	71	NA	16	10

Condor ID	DateColl[a]	Site[b]	CORT (ng/mL)	FlightPen (hr)	TechEntry (min)	MinSinceHandl (min)
650	10/14/2015	PNP	87	141	169	5
652	1/7/2014	LAZ	77	NA	37	4
663	5/28/2015	VWS	82	28	121	6
663	10/21/2015	PNP	131	23	160	6
684	6/23/2015	PNP	101	44	83	6
684	10/14/2015	PNP	66	45	131	6
687	10/7/2015	PNP	51	44	36	7
688	6/3/2015	VWS	13	26	151	4
692	6/10/2015	PNP	16	19	8	5
700	10/7/2015	PNP	40	119	13	5
704	10/21/2015	PNP	62	21	125	7
729	10/14/2015	PNP	92	119	68	5
745	5/28/2015	VWS	83	28	20	13
769	6/3/2015	VWS	55	24	68	6

[a]Date of handling event and sample collection. [b]Site of handling event and sample collection: LAZ = Los Angeles zoo and Botanical Gardens, SBZ = Santa Barbara Zoo, VWS = Ventana Wildlife Society in Big Sur, PNP = Pinnacles National Park. See Table 2 for definitions of remaining column headers.

Table S3. Urate samples and associated stressor variables. First urate sample in series for each condor was 'first urate GCM' response variable, GCM concentration (ng/g dry wt.) for 'peak urate GCM' within two hours of handling start is highlighted in gray for each urate series, and ∆urate is the difference in GCM concentration (ng/g dry wt.) between these two samples. See Table to for stressor variable definitions.

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
23	1	6/14/2016	LAZ	5.14	0.30	623	185	NA	151	22	22
23	2	6/14/2016	LAZ	0.40	0.03	478	12	NA	151	39	22
23	3	6/14/2016	LAZ	0.12	0.02	617	10	NA	151	57	22
23	4	6/14/2016	LAZ	0.22	0.02	1227	24	NA	151	66	22
23	5	6/14/2016	LAZ	0.15	0.01	1191	16	NA	151	83	22
23	6	6/14/2016	LAZ	0.24	0.03	862	29	NA	151	118	22
23	7	6/14/2016	LAZ	0.46	0.06	1101	67	NA	151	136	22
23	8	6/14/2016	LAZ	0.11	0.02	773	13	NA	151	155	22
23	9	6/14/2016	LAZ	0.57	0.06	1367	88	NA	151	187	22
23	10	6/14/2016	LAZ	0.11	0.01	1041	14	NA	151	214	22
23	12	6/14/2016	LAZ	1.57	0.20	860	171	NA	151	236	22
120	1	6/14/2016	LAZ	11.49	0.20	440	89	NA	68	28	28
120	2	6/14/2016	LAZ	1.48	0.04	342	12	NA	68	36	28
120	3	6/14/2016	LAZ	2.65	0.33	474	157	NA	68	59	28
120	4	6/14/2016	LAZ	1.92	0.21	341	70	NA	68	71	28
120	5	6/14/2016	LAZ	3.93	0.25	398	98	NA	68	86	28
120	6	6/14/2016	LAZ	2.20	0.11	482	55	NA	68	91	28

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
120	7	6/14/2016	LAZ	1.95	0.08	592	48	NA	68	96	28
120	8	6/14/2016	LAZ	4.07	0.13	507	68	NA	68	98	28
120	9	6/14/2016	LAZ	1.92	0.06	715	42	NA	68	113	28
120	10	6/14/2016	LAZ	4.17	0.18	569	101	NA	68	125	28
120	11	6/14/2016	LAZ	3.01	0.17	539	93	NA	68	140	28
120	12	6/14/2016	LAZ	2.15	0.11	509	55	NA	68	157	28
120	13	6/14/2016	LAZ	6.85	0.28	617	171	NA	68	170	28
120	14	6/14/2016	LAZ	0.03	0.00	104	1	NA	68	177	28
120	15	6/14/2016	LAZ	6.23	0.47	470	222	NA	68	186	28
120	16	6/14/2016	LAZ	4.69	0.37	410	152	NA	68	197	28
120	17	6/14/2016	LAZ	5.02	0.50	526	263	NA	68	215	28
120	18	6/14/2016	LAZ	2.65	0.25	499	125	NA	68	234	28
120	19	6/14/2016	LAZ	5.27	0.25	776	191	NA	68	238	28
120	20	6/14/2016	LAZ	1.69	0.14	195	27	NA	68	260	28
120	21	6/14/2016	LAZ	2.26	0.16	617	97	NA	68	273	28
120	22	6/14/2016	LAZ	1.65	0.12	799	97	NA	68	290	28
120	23	6/14/2016	LAZ	0.46	0.03	1061	37	NA	68	319	28
120	25	6/14/2016	LAZ	2.37	0.09	1387	122	NA	68	336	28
159	1	6/14/2016	LAZ	2.48	0.57	26	15	NA	126	22	21
159	2	6/14/2016	LAZ	0.71	0.21	112	23	NA	126	38	21
159	4+5	6/14/2016	LAZ	3.57	0.80	153	122	NA	126	57	21
159	6	6/14/2016	LAZ	0.80	0.11	249	27	NA	126	99	21
159	7	6/14/2016	LAZ	0.97	0.12	257	31	NA	126	112	21
159	9	6/14/2016	LAZ	1.04	0.09	281	24	NA	126	123	21
159	10	6/14/2016	LAZ	5.03	0.28	290	81	NA	126	142	21

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
159	12	6/14/2016	LAZ	1.22	0.06	200	12	NA	126	162	21
159	13	6/14/2016	LAZ	8.08	0.82	156	127	NA	126	170	21
159	14	6/14/2016	LAZ	5.11	0.39	331	129	NA	126	186	21
159	15	6/14/2016	LAZ	4.74	0.47	363	172	NA	126	218	21
159	17	6/14/2016	LAZ	0.64	0.04	497	22	NA	126	236	21
159	20	6/14/2016	LAZ	2.85	0.22	610	135	NA	126	265	21
159	22	6/14/2016	LAZ	0.11	0.02	292	7	NA	126	284	21
168	1	10/26/2015	VWS	5.48	0.07	757	54	98	52	30	30
168	3	10/26/2015	VWS	1.50	0.05	569	30	98	52	38	30
168	5	10/26/2015	VWS	2.24	0.09	1265	112	98	52	51	30
168	6	10/26/2015	VWS	0.31	0.02	802	14	98	52	58	30
168	7	10/26/2015	VWS	2.07	0.07	1634	112	98	52	68	30
168	9	10/26/2015	VWS	2.13	0.05	963	46	98	52	90	30
168	10	10/26/2015	VWS	4.37	0.08	1882	143	98	52	96	30
168	11	10/26/2015	VWS	4.91	0.08	1781	147	98	52	106	30
168	14	10/26/2015	VWS	3.96	0.08	3416	260	98	52	138	30
168	15	10/26/2015	VWS	2.94	0.07	3255	241	98	52	148	30
168	17	10/26/2015	VWS	3.48	0.12	2479	306	98	52	172	30
168	18	10/26/2015	VWS	11.78	0.18	2926	521	98	52	175	30
174	1	7/28/2016	SBZ	1.32	0.12	564	70	NA	2	25	25
174	3	7/28/2016	SBZ	0.40	0.05	840	42	NA	2	82	25
174	4	7/28/2016	SBZ	2.53	0.15	1053	159	NA	2	114	25
174	5	7/28/2016	SBZ	0.82	0.05	774	42	NA	2	141	25
174	6	7/28/2016	SBZ	0.20	0.02	533	13	NA	2	168	25
174	7	7/28/2016	SBZ	1.28	0.17	1232	214	NA	2	217	25

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
174	9	7/28/2016	SBZ	0.98	0.17	761	127	NA	2	279	25
174	11	7/28/2016	SBZ	0.08	0.02	4	0	NA	2	282	25
209	1	10/16/2014	VWS	3.02	0.04	1260	52	98	103	23	15
209	1	10/29/2015	VWS	3.56	0.18	1520	267	26	59	39	39
209	2	10/29/2015	VWS	0.82	0.04	2005	84	26	59	46	39
209	3	10/29/2015	VWS	0.67	0.05	1858	86	26	59	56	39
209	4	10/29/2015	VWS	0.13	0.02	904	20	26	59	64	39
209	5	10/29/2015	VWS	0.64	0.07	2288	153	26	59	73	39
209	6	10/29/2015	VWS	0.04	0.01	669	9	26	59	89	39
209	7	10/29/2015	VWS	0.17	0.02	1015	16	26	59	103	39
209	8	10/29/2015	VWS	1.15	0.04	2872	109	26	59	112	39
219	1	10/26/2015	VWS	8.37	0.15	641	96	98	53	22	21
219	2	10/26/2015	VWS	0.56	0.02	641	10	98	53	22	21
219	3	10/26/2015	VWS	0.60	0.02	705	17	98	53	39	21
219	4	10/26/2015	VWS	0.16	0.02	794	12	98	53	54	21
219	5	10/26/2015	VWS	0.31	0.04	848	31	98	53	59	21
219	6	10/26/2015	VWS	0.17	0.02	779	17	98	53	69	21
219	7	10/26/2015	VWS	0.02	0.02	167	3	98	53	75	21
219	8	10/26/2015	VWS	0.31	0.03	1242	37	98	53	81	21
219	9	10/26/2015	VWS	0.04	0.01	620	4	98	53	91	21
219	10	10/26/2015	VWS	0.02	0.01	179	2	98	53	98	21
219	11	10/26/2015	VWS	0.07	0.01	1497	11	98	53	108	21
219	12	10/26/2015	VWS	0.46	0.06	2004	114	98	53	125	21
219	13	10/26/2015	VWS	0.19	0.04	1306	53	98	53	144	21
219	15	10/26/2015	VWS	0.09	0.02	981	18	98	53	202	21

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
231	1	10/16/2014	VWS	3.30	0.16	1248	205	22	58	32	27
231	2	10/16/2014	VWS	1.65	0.03	795	23	22	58	40	27
231	3	10/16/2014	VWS	0.71	0.04	2069	83	22	58	58	27
231	4	10/16/2014	VWS	0.89	0.10	2131	210	22	58	76	27
231	5	10/16/2014	VWS	0.68	0.09	2363	206	22	58	89	27
231	6	10/16/2014	VWS	0.51	0.04	3882	171	22	58	100	27
231	7	10/16/2014	VWS	0.99	0.11	2946	311	22	58	132	27
231	9	10/16/2014	VWS	0.34	0.04	3384	135	22	58	175	27
231	10	10/16/2014	VWS	0.37	0.05	3111	143	22	58	187	27
231	11	10/16/2014	VWS	0.76	0.06	3061	186	22	58	220	27
340	1	10/14/2015	PNP	2.05	0.03	466	15	42	30	27	27
340	3	10/14/2015	PNP	2.32	0.07	530	37	42	30	32	27
340	4	10/14/2015	PNP	3.68	0.06	837	53	42	30	36	27
340	6	10/14/2015	PNP	2.47	0.05	1260	60	42	30	43	27
340	7	10/14/2015	PNP	0.07	0.01	359	4	42	30	56	27
340	8	10/14/2015	PNP	0.14	0.01	955	14	42	30	61	27
340	9	10/14/2015	PNP	0.23	0.03	1713	48	42	30	78	27
340	10	10/14/2015	PNP	0.04	0.02	490	8	42	30	81	27
340	11	10/14/2015	PNP	0.27	0.03	2354	81	42	30	89	27
340	12	10/14/2015	PNP	0.13	0.02	2201	38	42	30	98	27
340	13	10/14/2015	PNP	0.14	0.02	1872	45	42	30	125	27
340	14	10/14/2015	PNP	0.42	0.03	3310	103	42	30	127	27
340	15	10/14/2015	PNP	0.26	0.02	3983	61	42	30	142	27
340	16	10/14/2015	PNP	0.31	0.03	4065	123	42	30	162	27
340	17	10/14/2015	PNP	0.22	0.02	4609	94	42	30	171	27

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
351	1	6/10/2015	PNP	0.32	0.03	671	19	18	41	35	34
351	2	6/10/2015	PNP	2.73	0.08	879	70	18	41	41	34
351	4	6/10/2015	PNP	0.70	0.05	310	15	18	41	51	34
431	1	10/16/2014	VWS	4.79	0.04	452	18	23	23	31	31
431	2	10/16/2014	VWS	1.25	0.02	371	9	23	23	40	NA
448	1	6/16/2014	PNP	4.80	0.13	512	122	20	11	29	28
448	2	6/16/2014	PNP	1.41	0.22	720	162	20	11	67	28
448	3	6/16/2014	PNP	4.73	0.30	3794	190	20	11	99	28
448	4	6/16/2014	PNP	0.96	0.07	1029	73	20	11	111	28
448	5	6/16/2014	PNP	2.41	0.10	946	91	20	11	127	28
448	6	6/16/2014	PNP	1.33	0.05	2165	101	20	11	167	28
448	7	6/16/2014	PNP	2.95	0.16	642	106	20	11	177	28
463	1	10/28/2015	PNP	7.67	0.06	578	35	21	45	31	31
463	2	10/28/2015	PNP	1.19	0.06	466	26	21	45	34	31
463	3	10/28/2015	PNP	0.31	0.04	564	20	21	45	39	31
463	4	10/28/2015	PNP	0.26	0.03	492	17	21	45	41	31
463	5	10/28/2015	PNP	0.19	0.03	554	14	21	45	43	31
463	6	10/28/2015	PNP	0.27	0.03	819	26	21	45	45	31
463	8	10/28/2015	PNP	0.13	0.02	939	14	21	45	48	31
463	10	10/28/2015	PNP	0.82	0.09	1394	132	21	45	57	31
464	1	7/28/2016	SBZ	0.36	0.02	1413	33	NA	100	24	24
464	2	7/28/2016	SBZ	1.06	0.12	1576	192	NA	100	47	24
464	3	7/28/2016	SBZ	4.22	0.20	1143	233	NA	100	88	24
464	4	7/28/2016	SBZ	2.30	0.09	1279	121	NA	100	116	24
464	5	7/28/2016	SBZ	0.07	0.01	12	0	NA	100	120	24

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
464	6	7/28/2016	SBZ	1.36	0.09	4619	411	NA	100	163	24
464	7	7/28/2016	SBZ	0.75	0.10	1995	195	NA	100	187	24
464	8	7/28/2016	SBZ	1.70	0.19	2368	456	NA	100	245	24
470	1	10/29/2015	VWS	1.12	0.12	263	32	21	25	29	26
470	2	10/29/2015	VWS	0.50	0.02	2081	51	21	25	38	26
470	3	10/29/2015	VWS	0.07	0.01	1817	10	21	25	47	26
470	4	10/29/2015	VWS	0.03	0.01	1044	11	21	25	53	26
470	5	10/29/2015	VWS	0.07	0.01	3080	35	21	25	65	26
470	6	10/29/2015	VWS	0.16	0.02	5129	100	21	25	75	26
470	7	10/29/2015	VWS	0.22	0.03	5157	144	21	25	95	26
470	8	10/29/2015	VWS	0.39	0.05	4173	209	21	25	109	26
470	9	10/29/2015	VWS	0.50	0.05	6610	348	21	25	119	26
470	10	10/29/2015	VWS	0.27	0.04	4611	176	21	25	129	26
470	11	10/29/2015	VWS	0.25	0.03	7214	234	21	25	158	26
534	1	10/16/2014	VWS	10.46	0.10	772	76	22	143	30	30
534	2	10/16/2014	VWS	9.20	0.07	597	39	22	143	38	30
538	1	5/27/2015	PNP	2.29	0.11	850	97	91	5	44	44
538	2	5/27/2015	PNP	0.96	0.12	141	17	91	5	47	44
538	4	5/27/2015	PNP	0.11	0.01	555	4	91	5	54	44
538	5	5/27/2015	PNP	0.76	0.05	809	37	91	5	57	44
538	7	5/27/2015	PNP	1.80	0.04	1911	85	91	5	59	44
538	8	5/27/2015	PNP	1.18	0.03	3312	106	91	5	70	44
543	1	10/21/2015	PNP	7.27	0.53	615	326	23	80	31	31
543	3	10/21/2015	PNP	6.14	0.17	844	143	23	80	39	31
543	4	10/21/2015	PNP	3.24	0.08	797	64	23	80	52	31

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
543	5	10/21/2015	PNP	1.46	0.05	1168	63	23	80	55	31
543	6	10/21/2015	PNP	2.44	0.11	369	42	23	80	76	31
543	7	10/21/2015	PNP	0.63	0.05	935	47	23	80	88	31
543	9	10/21/2015	PNP	1.87	0.11	2116	228	23	80	94	31
543	10	10/21/2015	PNP	0.55	0.04	1857	69	23	80	107	31
544	1	7/28/2016	SBZ	2.55	0.10	472	45	NA	65	27	27
544	2	7/28/2016	SBZ	1.55	0.16	1182	194	NA	65	81	27
544	3	7/28/2016	SBZ	2.62	0.35	471	164	NA	65	177	27
544	4	7/28/2016	SBZ	1.09	0.12	1534	183	NA	65	222	27
544	7	7/28/2016	SBZ	0.67	0.20	433	87	NA	65	255	27
547	1	6/3/2015	VWS	2.98	0.06	722	41	26	93	18	18
547	3	6/3/2015	VWS	0.93	0.02	1089	18	26	93	28	18
547	4	6/3/2015	VWS	1.21	0.02	1111	27	26	93	40	18
547	5	6/3/2015	VWS	0.77	0.02	1377	24	26	93	43	18
547	7	6/3/2015	VWS	0.78	0.03	1710	44	26	93	73	18
547	8	6/3/2015	VWS	2.33	0.08	1231	100	26	93	93	18
547	9	6/3/2015	VWS	1.51	0.04	1640	66	26	93	96	18
547	10	6/3/2015	VWS	0.63	0.03	1077	29	26	93	115	18
547	11	6/3/2015	VWS	0.35	0.02	808	13	26	93	118	18
547	12	6/3/2015	VWS	0.94	0.02	1408	32	26	93	121	18
547	14	6/3/2015	VWS	0.98	0.04	1057	41	26	93	129	18
569	1	10/9/2014	VWS	3.57	0.05	954	47	16	7	28	28
569	2	10/9/2014	VWS	1.80	0.28	104	29	16	7	36	28
569	3	10/9/2014	VWS	1.35	0.11	6001	635	16	7	73	28
569	4	10/9/2014	VWS	0.24	0.02	1103	19	16	7	91	28

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
569	5	10/9/2014	VWS	0.32	0.01	1402	18	16	7	113	28
569	6	10/9/2014	VWS	0.35	0.04	1114	42	16	7	175	28
569	7	10/9/2014	VWS	0.11	0.02	825	13	16	7	195	28
569	8	10/9/2014	VWS	0.37	0.02	2635	65	16	7	209	28
569	9	10/9/2014	VWS	0.15	0.02	1676	27	16	7	218	28
583	1	10/16/2014	VWS	8.71	0.24	414	100	26	23	31	30
583	3	10/16/2014	VWS	11.92	0.10	734	71	26	23	36	30
583	5	10/16/2014	VWS	7.01	0.15	751	116	26	23	55	30
583	2	5/6/2015	PNP	3.13	0.28	911	252	23	29	33	34
583	3	5/6/2015	PNP	2.24	0.43	1227	524	23	29	77	34
583	5	5/6/2015	PNP	0.15	0.03	2390	71	23	29	82	34
606	1	4/9/2014	PNP	0.35	0.02	213	3	164	40	0	38
606	1	5/28/2015	VWS	12.33	0.28	620	173	26	53	40	40
606	3	5/28/2015	VWS	2.22	0.03	1500	46	26	53	42	40
606	4	5/28/2015	VWS	2.33	0.06	1581	95	26	53	60	40
606	5	5/28/2015	VWS	0.59	0.07	1464	104	26	53	76	40
606	6	5/28/2015	VWS	0.40	0.03	2073	66	26	53	90	40
606	7	5/28/2015	VWS	1.38	0.03	2307	78	26	53	105	40
606	8	5/28/2015	VWS	0.16	0.01	2011	28	26	53	126	40
606	9	5/28/2015	VWS	0.44	0.04	1455	53	26	53	139	40
606	11	5/28/2015	VWS	1.70	0.06	1683	105	26	53	142	40
606	1	10/21/2015	PNP	3.47	0.09	296	27	71	42	32	32
606	3	10/21/2015	PNP	1.43	0.03	268	9	71	42	43	32
606	4	10/21/2015	PNP	0.99	0.03	442	11	71	42	46	32
606	5	10/21/2015	PNP	0.99	0.02	484	9	71	42	51	32

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
606	6	10/21/2015	PNP	3.70	0.13	490	65	71	42	66	32
606	7	10/21/2015	PNP	0.70	0.05	974	47	71	42	69	32
606	8	10/21/2015	PNP	0.20	0.02	622	10	71	42	82	32
606	9	10/21/2015	PNP	0.42	0.06	519	31	71	42	95	32
606	10	10/21/2015	PNP	0.30	0.03	688	23	71	42	99	32
606	11	10/21/2015	PNP	0.32	0.03	1107	33	71	42	105	32
606	12	10/21/2015	PNP	0.88	0.04	550	20	71	42	123	32
606	13	10/21/2015	PNP	0.04	0.00	355	2	71	42	135	32
615	4	6/4/2014	PNP	0.91	0.02	938	21	117	21	48	34
615	8	6/4/2014	PNP	0.11	0.01	433	5	117	21	89	34
626	1	10/29/2014	PNP	1.91	0.05	2270	28	48	14	11	41
626	2	10/29/2014	PNP	1.08	0.02	330	7	48	14	41	41
626	3	10/29/2014	PNP	1.40	0.05	425	22	48	14	50	41
626	4	10/29/2014	PNP	0.41	0.03	458	12	48	14	51	41
626	5	10/29/2014	PNP	0.66	0.06	833	47	48	14	59	41
626	6	10/29/2014	PNP	0.62	0.06	875	51	48	14	73	41
626	7	10/29/2014	PNP	1.02	0.08	1540	131	48	14	93	41
626	8	10/29/2014	PNP	0.40	0.03	1343	44	48	14	103	41
626	10	10/29/2014	PNP	1.70	0.10	692	70	48	14	121	41
626	11	10/29/2014	PNP	0.22	0.03	1919	62	48	14	233	41
626	13	10/29/2014	PNP	0.29	0.04	2683	105	48	14	260	41
626	1	6/23/2015	PNP	12.20	0.18	2704	452	142	43	33	30
626	2	6/23/2015	PNP	1.38	0.06	2080	116	142	43	44	30
626	3	6/23/2015	PNP	1.06	0.11	937	103	142	43	68	30
626	4	6/23/2015	PNP	0.22	0.02	653	15	142	43	71	30

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
626	5	6/23/2015	PNP	0.66	0.06	991	56	142	43	87	30
626	1	10/29/2015	VWS	32.59	0.36	338	123	46	51	17	17
626	2	10/29/2015	VWS	5.12	0.04	626	27	46	51	29	17
626	4	10/29/2015	VWS	6.61	0.08	858	72	46	51	43	17
626	5	10/29/2015	VWS	2.90	0.04	906	40	46	51	51	17
626	6	10/29/2015	VWS	7.35	0.09	1218	106	46	51	62	17
626	7	10/29/2015	VWS	5.29	0.08	1405	117	46	51	81	17
626	8	10/29/2015	VWS	1.78	0.03	1546	54	46	51	93	17
626	9	10/29/2015	VWS	5.85	0.14	1752	241	46	51	108	17
626	10	10/29/2015	VWS	1.40	0.05	1916	94	46	51	119	17
626	11	10/29/2015	VWS	1.17	0.07	2232	149	46	51	154	17
626	12	10/29/2015	VWS	0.25	0.02	1477	26	46	51	171	17
631	2	2/25/2014	VWS	0.28	0.02	212	4	NA	43	29	20
631	7	2/25/2014	VWS	0.68	0.06	393	23	NA	43	108	20
631	9	2/25/2014	VWS	0.89	0.03	875	26	NA	43	120	20
631	1	6/3/2015	VWS	12.51	0.26	297	88	24	116	24	24
631	2	6/3/2015	VWS	2.70	0.15	513	77	24	116	49	24
631	3	6/3/2015	VWS	0.98	0.06	572	32	24	116	50	24
631	4	6/3/2015	VWS	1.75	0.15	458	71	24	116	81	24
631	5	6/3/2015	VWS	1.22	0.16	237	37	24	116	95	24
631	6	6/3/2015	VWS	4.21	0.10	1188	124	24	116	108	24
631	1	10/14/2015	PNP	27.30	0.29	274	79	141	7	21	21
631	3	10/14/2015	PNP	3.31	0.09	109	10	141	7	30	21
631	4	10/14/2015	PNP	0.53	0.01	1	0	141	7	32	21
631	5	10/14/2015	PNP	15.55	0.40	109	44	141	7	34	21

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
631	6	10/14/2015	PNP	12.91	0.31	132	41	141	7	41	21
631	7	10/14/2015	PNP	5.69	0.06	446	29	141	7	51	21
631	8	10/14/2015	PNP	4.53	0.08	502	38	141	7	53	21
631	9	10/14/2015	PNP	2.97	0.05	773	40	141	7	69	21
631	10	10/14/2015	PNP	1.05	0.02	700	13	141	7	78	21
631	11	10/14/2015	PNP	4.71	0.08	584	45	141	7	83	21
631	12	10/14/2015	PNP	5.20	0.08	1012	81	141	7	94	21
631	13	10/14/2015	PNP	3.40	0.06	1079	66	141	7	105	21
631	14	10/14/2015	PNP	1.96	0.07	1162	83	141	7	122	21
631	15	10/14/2015	PNP	2.90	0.07	1348	88	141	7	124	21
631	17	10/14/2015	PNP	1.94	0.06	1812	116	141	7	158	21
631	18	10/14/2015	PNP	0.57	0.03	1991	62	141	7	199	21
646	1	1/7/2014	LAZ	0.10	0.06	280	16	NA	71	294	20
650	1	2/25/2014	VWS	0.23	0.04	300	4	NA	90	25	25
650	4	2/25/2014	VWS	6.69	0.68	337	229	NA	90	77	25
652	2	10/23/2014	VWS	14.98	0.16	1860	312	25	45	32	25
652	4	10/23/2014	VWS	2.51	0.28	344	95	25	45	52	25
652	5	10/23/2014	VWS	2.28	0.08	555	46	25	45	61	25
652	6	10/23/2014	VWS	3.78	0.09	852	79	25	45	69	25
652	7	10/23/2014	VWS	4.70	0.10	851	83	25	45	79	25
652	8	10/23/2014	VWS	4.51	0.10	1151	112	25	45	88	25
652	9	10/23/2014	VWS	1.88	0.05	665	36	25	45	101	25
652	10	10/23/2014	VWS	2.33	0.06	829	50	25	45	109	25
652	11	10/23/2014	VWS	1.46	0.03	1082	34	25	45	111	25
652	12	10/23/2014	VWS	4.55	0.09	1070	101	25	45	114	25

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
652	13	10/23/2014	VWS	1.83	0.07	1031	76	25	45	134	25
652	15	10/23/2014	VWS	0.70	0.16	749	118	25	45	309	25
663	1	5/28/2015	VWS	1.85	0.15	304	45	28	121	31	30
663	2	5/28/2015	VWS	0.73	0.13	563	71	28	121	35	30
663	4	5/28/2015	VWS	0.89	0.14	1208	174	28	121	76	30
663	5	5/28/2015	VWS	0.09	0.02	799	18	28	121	79	30
684	1	6/23/2015	PNP	3.41	0.10	1013	105	44	83	22	22
684	2	6/23/2015	PNP	0.45	0.03	555	15	44	83	26	22
684	3	6/23/2015	PNP	2.74	0.18	872	158	44	83	34	22
684	4	6/23/2015	PNP	0.86	0.07	846	63	44	83	45	22
687	1	10/7/2015	PNP	0.30	0.02	1	0	44	36	33	35
687	2	10/7/2015	PNP	9.17	0.28	228	65	44	36	40	35
687	3	10/7/2015	PNP	9.27	0.16	482	77	44	36	47	35
687	4	10/7/2015	PNP	3.05	0.08	527	43	44	36	59	35
687	5	10/7/2015	PNP	8.02	0.27	468	124	44	36	69	35
687	7	10/7/2015	PNP	0.59	0.02	657	14	44	36	79	35
692	1	6/10/2015	PNP	3.54	0.16	109	17	19	8	26	22
692	2	6/10/2015	PNP	5.00	0.25	489	129	19	8	28	22
692	3	6/10/2015	PNP	2.13	0.06	171	11	19	8	34	22
692	4	6/10/2015	PNP	10.67	0.47	531	340	19	8	44	22
692	5	6/10/2015	PNP	5.11	0.25	133	36	19	8	50	22
692	6	6/10/2015	PNP	1.56	0.11	626	69	19	8	58	22
692	7	6/10/2015	PNP	1.02	0.12	165	20	19	8	61	22
692	8	6/10/2015	PNP	3.03	0.16	200	32	19	8	64	22
692	10	6/10/2015	PNP	2.97	0.16	996	163	19	8	77	22

Condor ID	Sample ID	Date Coll.[a]	Site[b]	g wet	g dry	GCM (ng/g dry wt.)	Total GCM (ng)	FlightPen (hr)	TechEntry (min)	MinSince Handl (min)	Restraint Dur (min)
692	2	6/10/2015	PNP	2.17	0.11	489	56	19	8	28	22
692	4	6/10/2015	PNP	5.46	0.33	531	174	19	8	44	22
692	5	6/10/2015	PNP	2.06	0.11	133	15	19	8	50	22
700	1	10/7/2015	PNP	2.43	0.05	362	20	119	13	22	20
700	2	10/7/2015	PNP	1.64	0.02	2	0	119	13	25	20
700	3	10/7/2015	PNP	3.39	0.07	359	26	119	13	28	20
700	4	10/7/2015	PNP	3.66	0.11	268	29	119	13	33	20
700	6	10/7/2015	PNP	0.66	0.06	423	27	119	13	58	20
700	7	10/7/2015	PNP	0.90	0.07	682	45	119	13	65	20
700	8	10/7/2015	PNP	1.43	0.09	1347	120	119	13	89	20
745	1	5/28/2015	VWS	11.66	0.16	517	84	28	20	27	27
745	2	5/28/2015	VWS	2.24	0.06	913	53	28	20	42	27
745	4	5/28/2015	VWS	1.40	0.08	1029	87	28	20	66	27
745	5	5/28/2015	VWS	0.89	0.05	1157	54	28	20	80	27
745	6	5/28/2015	VWS	0.20	0.02	575	11	28	20	89	27
745	7	5/28/2015	VWS	1.09	0.20	587	118	28	20	119	27

[a]Date of handling event and sample collection. [b]Site of handling event and sample collection: LAZ = Los Angeles zoo and Botanical Gardens, SBZ = Santa Barbara Zoo, VWS = Ventana Wildlife Society in Big Sur, PNP = Pinnacles National Park. See Table 2 for definitions of remaining column headers.

Table S4. Top models from first model selection step for wild and captive condor GC response variable prediction.

Plasma top model (Wild and Captive):

Parameter	Estimate	SE	p value	R^2	N
Intercept	55.994	12.3	3.6 e-05	0.1673	52
Age	-4.479	14.293	0.7554		
Status	26.083	13.736	0.0636		
age:status(*wild)*	49.778	23.175	0.0368		

First urate top model (Wild and Captive):

Parameter	Estimate	SE	p value	R^2	N
Intercept	658	75.8	1.50e-10	0.1055	40
t since handling start	322.3	152.3	0.0409		

Peak urate top model (Wild and Captive):

Parameter	Estimate	SE	p value	R^2	N
Intercept	2002.5	222.3	2.74e-10	0.1817	34
t tech entry	-1139.8	427.7	0.012		

∆ urate GCM top model (Wild and Captive):

Parameter	Estimate	SE	p value	R^2	N
Intercept	1319.6	216.6	8.28e-07	0.1774	34
t tech entry	-1094.5	416.6	0.0131		

Table S5. All models considered in first round of model selection step with intrinsic and environmental paramters for prediction of wild condor GC response outcomes. Selection criteria for candidate models was to be within summed AIC_c wt of 0.90 and >1 ΔAIC_c from intercept-only model.

Model Structure	n	K[a]	Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Sum w[e]	Evidence ratio[f]
Plasma CORT								
Age	41	3	-207.41	421.47	0.00	0.41	0.62	1.00
intercept	41	5	-205.03	421.77	0.29	0.35	0.74	1.16
FlightPen	41	2	-210.25	424.81	3.33	0.08	0.79	5.29
TechEntry	41	3	-209.99	426.62	5.15	0.03	0.83	13.11
Sex	41	3	-210.10	426.86	5.38	0.03	0.87	14.76
MinSinceHandl	41	3	-210.13	426.90	5.43	0.03	0.91	15.09
Season	41	3	-210.15	426.95	5.47	0.03	0.95	15.43
FlightPen + MinSinceHandl	41	3	-210.23	427.11	5.63	0.02	0.96	16.73
FlightPen + TechEntry	41	4	-209.87	428.85	7.38	0.01	0.98	40.00
TechEntry + MinSinceHandl	41	4	-209.95	429.01	7.53	0.01	0.99	43.21
FlightPen + TechEntry + MinSinceHandl	41	5	-209.62	430.95	9.48	0.00	1.00	114.29
global model	41	8	-206.27	433.03	11.56	0.00	1.00	323.22
First Urate GCM								
MinSinceHandl	33	3	-250.52	507.86	0.00	0.28	0.23	1.00
FlightPen + MinSinceHandl	33	2	-252.50	509.39	1.53	0.13	0.44	2.15
intercept	33	3	-251.42	509.66	1.80	0.11	0.55	2.46
Age	33	4	-250.34	510.11	2.25	0.09	0.64	3.08

Model Structure	n	K[a]	Log L	AIC$_c$[b]	ΔAIC$_c$[c]	w$_i$[d]	Sum w[e]	Evidence ratio[f]
First Urate GCM cont'd								
TechEntry + MinSinceHandl	33	5	-248.95	510.13	2.26	0.09	0.72	3.10
FlightPen + TechEntry + MinSinceHandl	33	3	-251.77	510.36	2.50	0.08	0.79	3.48
Sex	33	3	-252.22	511.27	3.40	0.05	0.86	5.48
FlightPen	33	3	-252.31	511.45	3.58	0.05	0.90	6.00
Season	33	5	-249.62	511.46	3.60	0.05	0.94	6.04
TechEntry	33	3	-252.33	511.49	3.63	0.05	0.98	6.15
FlightPen + TechEntry	33	4	-251.97	513.37	5.51	0.02	0.99	15.69
global model	33	4	-252.22	513.86	6.00	0.01	1.00	20.04
PeakUrate GCM								
FlightPen + TechEntry	27	4	-230.11	470.03	0.00	0.34	0.34	1.00
TechEntry	27	3	-232.05	471.14	1.11	0.20	0.53	1.74
FlightPen + TechEntry + RestraintDur	27	5	-229.85	472.55	2.52	0.10	0.63	3.52
intercept	27	2	-234.20	472.90	2.87	0.08	0.71	4.19
TechEntry + RestraintDur	27	4	-231.61	473.05	3.02	0.08	0.79	4.52
Age	27	3	-233.53	474.10	4.07	0.04	0.83	7.66
FlightPen	27	3	-233.57	474.19	4.16	0.04	0.87	8.00
RestraintDur	27	3	-233.61	474.26	4.23	0.04	0.91	8.28
Sex	27	3	-233.76	474.57	4.54	0.04	0.95	9.68
Season	27	3	-234.15	475.35	5.31	0.02	0.97	14.26
FlightPen + RestraintDur	27	4	-233.07	475.96	5.93	0.02	0.99	19.37
global model	27	8	-226.68	477.37	7.34	0.01	1.00	39.22

Model Structure	n	K[a]	Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Sum w[e]	Evidence ratio[f]
ΔUrate GCM								
FlightPen + TechEntry	27	4	-228.92	467.67	0.00	0.57	0.57	1.00
FlightPen + TechEntry + RestraintDur	27	5	-228.89	470.64	2.97	0.13	0.70	4.42
TechEntry	27	3	-231.94	470.92	3.25	0.11	0.81	5.08
intercept	27	2	-234.14	472.78	5.11	0.04	0.86	12.88
FlightPen	27	3	-233.02	473.08	5.41	0.04	0.89	14.96
TechEntry + RestraintDur	27	4	-231.79	473.39	5.73	0.03	0.93	17.51
Age	27	3	-233.79	474.63	6.96	0.02	0.94	32.51
RestraintDur	27	3	-233.87	474.78	7.12	0.02	0.96	35.12
Sex	27	3	-234.08	475.20	7.53	0.01	0.97	43.23
Season	27	3	-234.12	475.29	7.63	0.01	0.99	45.27
FlightPen + RestraintDur	27	4	-232.83	475.47	7.81	0.01	1.00	49.55
global model	27	8	-227.22	478.45	10.78	0.00	1.00	219.38

[a]Number of estimated parameters in the model including intercept and variance. [b]Second-order Akaike's information criterion (AIC), optimized for small sample size. [c]Difference in AIC_c value from that of most parsimonious model (i.e. model with lowest AIC_c). [d]Likelihood of the model relative to other models in the candidate set. [d]Weight of evidence that the top model is better than another model, given the candidate set. [e]Cumulative weights summed from best model to least supported model. [f] Likelihood that top model is better than model i.

Table S6. Top models from first model selection step for wild condor GC response variable prediction.

Plasma top model (Wild Only):

Parameter	Estimate	SE	p value	R^2	N
Intercept	78.146	6.099	1.48e-15	0.129	41
Age	29.686	12.35	0.0211		

First urate top model (Wild Only):

Parameter	Estimate	SE	p value	R^2	N
Intercept	684.1	86.1	5.71e-09	0.1131	33
MinSinceHandl	347.6	174.9	0.0557		

Peak urate top model (Wild Only):

Parameter	Estimate	SE	p value	R^2	N
Intercept	2231.2	248.8	3.94e-09	0.2615	27
TechEntry	-1339.8	505.4	0.014		
FlightPen	-1101.8	571.9	0.066		

Δ urate GCM top model (Wild Only):

Parameter	Estimate	SE	p value	R^2	N
Intercept	1508.7	238.1	1.50e-06	0.3204	30
TechEntry	-1410.3	483.7	0.00758		
FlightPen	-1340.8	547.4	0.02198		

Table S7. All models considered in 2st round of model selection step with lead exposure and behavioral variables included for prediction of wild condor GC response outcomes. Selection criteria for candidate models was to be within summed AIC_c wt of 0.90 and >1 ΔAIC_c from intercept-only model.

Model Structure	n	K[a]	Log L	AIC_c[b]	ΔAIC_c[c]	w_i[d]	Sum w[e]	Evidence ratio[f]
Plasma CORT								
DaysAbsent	41	3	-203.37	413.40	0.00	0.50	0.50	1.00
Age + DaysAbsent	41	4	-202.56	414.23	0.83	0.33	0.84	1.51
YrsMMFed	41	3	-205.31	417.27	3.87	0.07	0.91	6.94
Age + YrsMMFed	41	4	-205.06	419.23	5.83	0.03	0.94	18.49
Age + DaysAbsent18	41	4	-205.54	420.18	6.79	0.02	0.95	29.74
Age + CurrentPb	41	4	-205.62	420.36	6.96	0.02	0.97	32.47
Age	41	3	-207.41	421.47	8.08	0.01	0.98	56.77
DaysAbsent18	41	3	-207.59	421.82	8.42	0.01	0.99	67.43
Age + PropHiPb18	41	4	-206.99	423.09	9.69	0.00	0.99	127.00
CurrentPb	41	3	-208.60	423.84	10.44	0.00	0.99	185.38
Age + PropHiPb	41	4	-207.41	423.93	10.53	0.00	1.00	193.72
global model	41	9	-200.39	424.58	11.18	0.00	1.00	268.00
intercept	41	2	-210.25	424.81	11.41	0.00	1.00	300.32
PropHiPb18	41	3	-210.09	426.84	13.44	0.00	1.00	828.66
PropHiPb	41	3	-210.19	427.03	13.63	0.00	1.00	913.62

Model Structure	n	K[a]	Log L	AIC$_c$[b]	ΔAIC$_c$[c]	w$_i$[d]	Sum w[e]	Evidence ratio[f]
First Urate GCM								
MinSinceHandl	33	3	-250.52	507.86	0.00	0.19	0.19	1.00
MinSinceHandl + PropHiPb	33	4	-249.63	508.69	0.82	0.13	0.32	1.51
intercept	33	2	-252.50	509.39	1.53	0.09	0.41	2.15
MinSinceHandl+ DaysAbsent	33	4	-250.15	509.73	1.87	0.07	0.48	2.55
MinSinceHandl + PropHiPb18	33	4	-250.26	509.94	2.08	0.07	0.55	2.82
PropHiPb	33	3	-251.59	510.01	2.14	0.07	0.61	2.92
MinSinceHandl + YrsMMFed	33	4	-250.31	510.05	2.19	0.06	0.68	2.99
DaysAbsent	33	3	-251.63	510.10	2.23	0.06	0.74	3.05
MinSinceHandl+ CurrentPb	33	4	-250.36	510.15	2.28	0.06	0.80	3.13
MinSinceHandl+ DaysAbsent18	33	4	-250.46	510.34	2.48	0.06	0.86	3.46
DaysAbsent18	33	3	-252.05	510.92	3.06	0.04	0.90	4.61
YrsMMFed	33	3	-252.06	510.95	3.09	0.04	0.94	4.68
PropHiPb18	33	3	-252.28	511.39	3.53	0.03	0.97	5.83
CurrentPb	33	3	-252.37	511.56	3.70	0.03	1.00	6.36
global model	33	9	-249.02	523.86	15.99	0.00	1.00	2970.40
PeakUrate GCM								
FlightPen + TechEntry + DaysAbsent	27	5	-226.85	466.55	0.00	0.38	0.38	1.00
FlightPen + TechEntry + YrsMMFed	27	5	-227.00	466.87	0.31	0.33	0.71	1.17
FlightPen + TechEntry + DaysAbsent18	27	5	-228.24	469.33	2.78	0.10	0.80	4.01
FlightPen + TechEntry	27	4	-230.11	470.03	3.48	0.07	0.87	5.69
YrsMMFed	27	3	-232.27	471.59	5.04	0.03	0.90	12.41
FlightPen + TechEntry + CurrentPb	27	5	-229.97	472.81	6.25	0.02	0.92	22.80
intercept	27	2	-234.20	472.90	6.34	0.02	0.93	23.85
DaysAbsent	27	3	-232.93	472.90	6.35	0.02	0.95	23.87

Model Structure	n	K[a]	Log L	AIC$_c$[b]	ΔAIC$_c$[c]	w$_i$[d]	Sum w[e]	Evidence ratio[f]
PeakUrate GCM cont'd								
FlightPen + TechEntry + PropHiPb	27	5	-230.03	472.91	6.36	0.02	0.96	24.06
FlightPen + TechEntry + PropHiPb18	27	5	-230.11	473.07	6.51	0.01	0.98	25.97
DaysAbsent18	27	3	-233.83	474.70	8.14	0.01	0.99	58.61
CurrentPb	27	3	-234.07	475.18	8.63	0.01	0.99	74.76
PropHiPb18	27	3	-234.19	475.43	8.88	0.00	1.00	84.63
PropHiPb	27	3	-234.20	475.44	8.89	0.00	1.00	85.05
global model	27	10	-225.40	484.55	18.00	0.00	1.00	8087.10
Delta Urate GCM								
FlightPen + TechEntry + YrsMMFed	27	5	-225.98	464.82	0.00	0.37	0.37	1.00
FlightPen + TechEntry + DaysAbsent	27	5	-226.11	465.07	0.26	0.32	0.69	1.14
FlightPen + TechEntry + DaysAbsent18	27	5	-227.12	467.09	2.27	0.12	0.81	3.12
FlightPen + TechEntry	27	4	-228.92	467.67	2.85	0.09	0.90	4.16
FlightPen + TechEntry + CurrentPb	27	5	-228.53	469.92	5.10	0.03	0.93	12.83
FlightPen + TechEntry + PropHiPb18	27	5	-228.91	470.68	5.86	0.02	0.95	18.74
FlightPen + TechEntry + PropHiPb	27	5	-228.92	470.70	5.89	0.02	0.97	18.98
YrsMMFed	27	3	-232.46	471.97	7.15	0.01	0.98	35.73
intercept	27	2	-234.14	472.78	7.96	0.01	0.99	53.54
DaysAbsent	27	3	-233.25	473.54	8.72	0.00	0.99	78.34
DaysAbsent18	27	3	-233.87	474.79	9.97	0.00	0.99	146.16
CurrentPb	27	3	-233.90	474.83	10.02	0.00	1.00	149.79
PropHiPb	27	3	-234.07	475.19	10.37	0.00	1.00	178.68
PropHiPb18	27	3	-234.08	475.21	10.39	0.00	1.00	180.35
global model	27	10	-224.41	482.57	17.75	0.00	1.00	7160.82

Notes for Table S7

[a]Number of estimated parameters in the model including intercept and variance. [b]Second-order Akaike's information criterion (AIC), optimized for small sample size. [c]Difference in AIC_c value from that of most parsimonious model (i.e. model with lowest AIC_c). [d]Likelihood of the model relative to other models in the candidate set. [d]Weight of evidence that the top model is better than another model, given the candidate set. [e]Cumulative weights summed from best model to least supported model. [f] Likelihood that top model is better than model i.

Table S8. Model averaged effects of top variables predicting GC response outcomes.

Plasma CORT					**90% CI[e]**	
Parameter	***N-models[a]***	***Sum wt.[b]***	**Estimate[c]**	**SE[d]**	**Lower**	**Upper**
Intercept	--	--	78.15	5.53	69.04	87.25
DaysAbsent	3	0.93	53.14	19.63	20.86	85.42
Age	2	0.45	-28.91	22.51	-65.94	8.11
YrsMMFed	2	0.20	27.98	23.79	-11.15	67.1

First urate GCM					**90% CI[e]**	
Parameter	**N-models[a]**	**Sum wt.[b]**	**Estimate[c]**	**SE[d]**	**Lower**	**Upper**
Intercept	--	--	684	87	540	828
MinSinceHandl	1	0.68	348	175	60	635

Peak urate GCM					**90% CI[e]**	
Parameter	**N-models[a]**	**Sum wt.[b]**	**Estimate[c]**	**SE[d]**	**Lower**	**Upper**
intercept	--	--	2220	232	1839	2600
FlightPen	5	0.96	-1152	533	-2028	-275
TechEntry	5	0.96	-1491	478	-2277	-705
DaysAbsent	2	0.51	1089	586	125	2054
YrsMMFed	2	0.47	1095	520	240	1950
DaysAbsent18	2	0.17	846	560	-75	1767

∆urate GCM					**90% CI[e]**	
Parameter	**N-models[a]**	**Sum wt.[b]**	**Estimate[c]**	**SE[d]**	**Lower**	**Upper**
intercept	--	--	1497	223	1131	1864
FlightPen	5	0.99	-1378	515	-2226	-530
TechEntry	5	0.99	-1542	463	-2303	-781
YrsMMFed	2	0.42	1045	500	222	1868
DaysAbsent	2	0.37	933	648	-133	2000
DaysAbsent18	2	0.15	825	523	-35	1684

[a]Number of competitive models including the variable. [b]Summed Akaike weights for all models with variable. [c]Weighted average beta coefficient. [d]Model averaged standard error. [e]90% confidence interval for beta coefficient estimate

Literature Cited in Appendix

Bortolotti, G.R., Marchant, T., Blas, J., Cabezas, S., 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. J. Exp. Biol. 212, 1477–82. doi:10.1242/jeb.022152

Cade, T. J., 2007. Exposure of California condors to lead from spent ammunition. J.Wildl. Manage. 71, 2125-2133.

Church, M. E., Gwiazda, R., Risebrough, R. W., Sorenson, K., Chamberlain, C. P., Farry, S., et al., 2006. Ammunition is the principal source of lead accumulated by California Condors re-introduced to the wild. Environ. Sci. Technol. 40, 6143-6150.

Finkelstein, M. E., George, D., Scherbinski, S., Gwiazda, R., Johnson, M., Burnett, J., et al., 2010. Feather Lead Concentrations and Pb-207/Pb-206 Ratios Reveal Lead Exposure History of California Condors (Gymnogyps californianus). Environ. Sci. Technol. 44, 2639-2647.

Rideout, B. A., Stalis, I., Papendick, R., Pessier, A. P., Puschner, B., Finkelstein, M. E., et al., 2012. Patterns of mortality in free-ranging California condors (Gymnogyps californianus). J. Wildl. Dis. 48, 95–112.

BIBLIOGRAPHY

Agency for Toxic Substances and Disease Registry, 2007. ToxGuide for Lead (Pb).

Aguilar, R.F., Yoshicedo, J.N., Parish, C.N., 2012. Ingluviotomy tube placement for lead-induced crop stasis in the California condor (*Gymnogyps californianus*). J. Avian Med. Surg. 26, 176–81.

Albano, N., Santiago-Quesada, F., Masero, J.A., Sánchez-Guzmán, J.M., Möstl, E., 2015. Immunoreactive cortisone in droppings reflect stress levels, diet and growth rate of gull-billed tern chicks. Gen. Comp. Endocrinol. 213, 74–80. doi:10.1016/j.ygcen.2015.02.019

Arnemo, J.M., Andersen, O., Stokke, S., Thomas, V.G., Krone, O., Pain, D.J., Mateo, R., 2016. Health and environmental risks from lead-based ammunition: science versus socio-politics. Ecohealth 13, 618–622. doi:10.1007/s10393-016-1177-x

Arnold, T.W., 2010. Uninformative parameters and model selection using Akaike's Information Criterion. J. Wildl. Manage. 74, 1175–1178. doi:10.2193/2009-367

Bakker, V.J., Smith, D.R., Copeland, H., Brandt, J., Wolstenholme, R., Burnett, J., Kirkland, S., Finkelstein, M.E., 2016. Effects of lead exposure, flock behavior, and management actions on the survival of California condors (*Gymnogyps californianus*). Ecohealth 1–14. doi:10.1007/s10393-015-1096-2

Baos, R., Blas, J., Bortolotti, G.R., Marchant, T. a., Hiraldo, F., 2006. Adrenocortical response to stress and thyroid hormone status in free-living nestling white storks (*Ciconia ciconia*) exposed to heavy metal and arsenic contamination. Environ. Health Perspect. 114, 1497–1501. doi:10.1289/ehp.9099

Baugh, A.T., Oers, K. van, Dingemanse, N.J., Hau, M., 2014. Baseline and stress-induced glucocorticoid concentrations are not repeatable but covary within individual great tits (*Parus major*). Gen. Comp. Endocrinol. 208, 154–163. doi:10.1016/j.ygcen.2014.08.014

Bellinger, D.C., Burger, J., Cade, T.J., Cory-Slechta, D.A., Finkelstein, M., Hu, H., Kosnett, M., Landrigan, P.J., Lanphear, B., Pokras, M.A., Redig, P.T., Rideout, B.A., Silbergeld, E., Wright, R., Smith, D.R., 2013. Health risks from lead-based ammunition in the environment. Environ. Health Perspect. 121. doi:10.1289/ehp.1306945

Benoff, S., Jacob, A., Hurley, I.R., 2000. Male infertility and environmental exposure to lead and cadmium. Hum. Reprod. Update 6, 107–21.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. Proc. Natl. Acad. Sci. U. S. A. 104, 8880–8884.

Bortolotti, G.R., Marchant, T. a., Blas, J., German, T., 2008. Corticosterone in feathers is a long-term, integrated measure of avian stress physiology. Funct. Ecol. 22, 494–500. doi:10.1111/j.1365-2435.2008.01387.x

Bortolotti, G.R., Marchant, T., Blas, J., Cabezas, S., 2009. Tracking stress: localisation, deposition and stability of corticosterone in feathers. J. Exp. Biol. 212, 1477–82. doi:10.1242/jeb.022152

Braun, E.J., 2014. Osmoregulatory Systems of Birds, Sturkie's Avian Physiology: Sixth Edition. Elsevier. doi:10.1016/B978-0-12-407160-5.00012-9

Breuner, C., 2011. Stress and reproduction in birds. Horm. Reprod. Vertebr. 129–151.

Breuner, C.W., Orchinik, M., 2009. Pharmacological characterization of intracellular, membrane, and plasma binding sites for corticosterone in house sparrows. Gen. Comp. Endocrinol. 163, 214–224. doi:10.1016/j.ygcen.2009.01.027

Bridge, E.S., Schoech, S.J., Bowman, R., Wingfield, J.C., 2009. Temporal predictability in food availability: effects upon the reproductive axis in Scrub-Jays. J. Exp. Zool. A. Ecol. Genet. Physiol. 311, 35–44. doi:10.1002/jez.493

Burnett, L.J., Sorenson, K.J., Brandt, J., Sandhaus, E.A., Ciani, D., Clark, M., David, C., Theule, J., Kasielke, S., Risebrough, R.W., 2013. Eggshell thinning and depressed hatching success of California condors reintroduced to central California. Condor 115, 477–491. doi:10.1525/cond.2013.110150

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Interference: A Practical Information-Theoretic Approach, 2nd ed. Springer-Verlag, New York.

Busch, D.S., 2010. Measuring stress in conservation settings: A reply to Linklater. Biol. Conserv. 143, 1039–1040. doi:10.1016/j.biocon.2009.12.028

Busch, D.S., Hayward, L.S., 2009. Stress in a conservation context: a discussion of glucocorticoid actions and how levels change with conservation-relevant variables. Biol. Conserv. 142, 2844–2853. doi:10.1016/j.biocon.2009.08.013

Buttler, E.I., Gilchrist, H.G., Descamps, S., Forbes, M.R., Soos, C., 2011. Handling stress of female common eiders during avian cholera outbreaks. J. Wildl. Manage. 75, 283–288. doi:10.1002/jwmg.38

Cade, T.J., 2007. Exposure of California condors to lead from spent ammunition. J. Wildl. Manage. 71, 2125. doi:10.2193/2007-084

Carsia, R. V., 2015. Adrenals, Sixth Edit. ed, Sturkie's Avian Physiology. Elsevier. doi:10.1016/B978-0-12-407160-5.00026-9

Chen, L., Wang, X., Zhang, X., Lam, P.K.S., Guo, Y., Lam, J.C.W., Zhou, B., 2017. Transgenerational endocrine disruption and neurotoxicity in zebrafish larvae after parental exposure to binary mixtures of decabromodiphenyl ether (BDE-209) and lead. Environ. Pollut. 230, 96–106. doi:10.1016/j.envpol.2017.06.053

Church, M.E., Gwiazda, R., Risebrough, R.W., Sorenson, K., Chamberlain, C.P., Farry, S., Heinrich, W., Rideout, B. a, Smith, D.R., 2006. Ammunition is the principal source of lead accumulated by California condors re-introduced to the wild. Environ. Sci. Technol. 40, 6143–50.

Collins, C., Kays, R., 2011. Causes of mortality in North American populations of large and medium-sized mammals. Anim. Conserv. 14, 474–483. doi:10.1111/j.1469-1795.2011.00458.x

Conde-Sieira, M., Valente, L.M.P., Hernández-Pérez, J., Soengas, J.L., Míguez, J.M., Gesto, M., 2018. Short-term exposure to repeated chasing stress does not induce habituation in Senegalese sole, *Solea senegalensis*. Aquaculture 487, 32–40. doi:10.1016/j.aquaculture.2018.01.003

Cornelius, J.M., Breuner, C.W., Hahn, T.P., 2012. Coping with the extremes: stress physiology varies between winter and summer in breeding opportunists. Biol. Lett. 8, 312–5. doi:10.1098/rsbl.2011.0865

Cory-Slechta, D.A., Virgolini, M.B., Rossi-George, A., Thiruchelvam, M., Lisek, R., Weston, D., 2008. Lifetime consequences of combined maternal lead and stress. Basic Clin. Pharmacol. Toxicol. 102, 218–27. doi:10.1111/j.1742-7843.2007.00189.x

Cyr, N.E., Michael Romero, L., 2007. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. Gen. Comp. Endocrinol. 151, 82–9. doi:10.1016/j.ygcen.2006.12.003

Davies, P.T., Sturge-Apple, M.L., Cicchetti, D., Cummings, E.M., 2007. The role of child adrenocortical functioning in pathways between interparental conflict and child maladjustment. Dev. Psychol. 43, 918–30. doi:10.1037/0012-1649.43.4.918

Denbow, D.M., Scanes, C.G., Carsia, R. V, Harvey, S., 2000. Sturkie's Avian Physiology, 5th ed. Academic Press, San Diego.

Deviche, P., Gao, S., Davies, S., Sharp, P.J., Dawson, A., 2012. Rapid stress-induced inhibition of plasma testosterone in free-ranging male rufous-winged sparrows, Peucaea carpalis: characterization, time course, and recovery. Gen. Comp. Endocrinol. 177, 1–8. doi:10.1016/j.ygcen.2012.02.022

Dickens, M.J., Earle, K. a, Romero, L.M., 2009. Initial transference of wild birds to captivity alters stress physiology. Gen. Comp. Endocrinol. 160, 76–83. doi:10.1016/j.ygcen.2008.10.023

Ecke, F., Singh, N.J., Arnemo, J.M., Bignert, A., Helander, B.B., Berglund, Å.M.M.M., Borg, H., Bröjer, C., Holm, K., Lanzone, M., Miller, T., Nordström, Å., Räikkönen, J., Rodushkin, I., Ågren, E., Hörnfeldt, B., Bro, Å.C., Holm, K., Lanzone, M., Miller, T., Nordstro, Å., Ra, J., Rodushkin, I., Ågren, E., Ho, B., 2017. Sublethal lead exposure alters movement behavior in free-ranging golden eagles. Environ. Sci. Technol. doi:10.1021/acs.est.6b06024

Ellenberg, U., Setiawan, A.N., Cree, A., Houston, D.M., Seddon, P.J., 2007. Elevated hormonal stress response and reduced reproductive output in Yellow-eyed penguins exposed to unregulated tourism. Gen. Comp. Endocrinol. 152, 54–63. doi:10.1016/j.ygcen.2007.02.022

Ellison, P.T., 2003. Energetics and reproductive effort. Am. J. Hum. Biol. 15, 342–51. doi:10.1002/ajhb.10152

Ferreyra, H., Beldomenico, P.M., Marchese, K., Romano, M., Caselli, A., Correa, A.I., Uhart, M., 2015. Lead exposure affects health indices in free-ranging ducks in Argentina. Ecotoxicology 24, 735–745. doi:10.1007/s10646-015-1419-7

Finkelstein, M., Kuspa, Z., Snyder, N.F., Schmitt, N.J., 2015. California Condor (*Gymnogyps californianus*). Birds North Am. doi:10.2173/bna.610

Finkelstein, M.E., Kuspa, Z.E., Welch, A., Eng, C., Clark, M., Burnett, J., Smith, D.R., 2014. Linking cases of illegal shootings of the endangered California condor using stable lead isotope analysis. Environ. Res. 134, 270–279. doi:10.1016/j.envres.2014.07.022

Finkelstein, M.E., Doak, D.F., George, D., Burnett, J., Brandt, J., Church, M., Grantham, J., Smith, D.R., 2012. Lead poisoning and the deceptive recovery of the critically endangered California condor. Proc. Natl. Acad. Sci. U. S. A. 109, 11449–54. doi:10.1073/pnas.1203141109

Finkelstein, M.E., George, D., Scherbinski, S., Gwiazda, R., Johnson, M., Burnett, J., Brandt, J., Lawrey, S., Pessier, A.P., Clark, M., Wynne, J., Grantham, J., Smith, D.R., 2010. Feather lead concentrations and (207)Pb/(206)Pb ratios reveal lead exposure history of California Condors (*Gymnogyps californianus*). Environ. Sci. Technol. 44, 2639–47. doi:10.1021/es903176w

Fisher, I.J., Pain, D.J., Thomas, V.G., 2006. A review of lead poisoning from ammunition sources in terrestrial birds. Biol. Conserv. 131, 421–432. doi:10.1016/j.biocon.2006.02.018

Fortin, M.C., Cory-Slechta, D.A., Ohman-Strickland, P., Yanger, T.S., Todd, A.C., Moynihan, J., Walton, J., Brooks, A., Fiedler, N., Nwankwo, C., Cory, D.A., Johnson, W., 2012. Increased lead biomarker levels are associated with changes in hormonal response to stress in occupationally exposed male participants. Environ. Health Perspect. 120, 278–283.

Fullmers, C.S., Edelsteinp, S., 1985. Lead-binding Properties of Intestinal Calcium-binding Proteins. J. Biol. Chem. 260, 6816–6819.

Gelman, A., 2008. Scaling regression inputs by dividing two standard deviations. Stat. Med. 27, 2865–2873. doi:10.1002/sim

Grémillet, D., Prudor, A., le Maho, Y., Weimerskirch, H., 2012. Vultures of the seas: hyperacidic stomachs in wandering albatrosses as an adaptation to dispersed food resources, including fishery wastes. PLoS One 7, e37834. doi:10.1371/journal.pone.0037834

Gump, B.B., Stewart, P., Reihman, J., Lonky, E., Darvill, T., Patrick, J., Granger, D.A., Parsons, P.J., 2008. Children's low-level prenatal and postnatal blood lead exposure and adrenocortical responses to acute stress in children. Environ. Health Perspect. 116, 249–255. doi:10.1289/ehp.l0391

Guyton, A.C., Hall, J.E., 2006. Textbook of Medical Physiology, 11th ed. ElsevierInc., Philadelphia.

Harvey, S., Phillips, J.G., Rees, A., Hall, T.R., 1984. Stress and adrenal function. J. Exp. Zool. 232, 633–45. doi:10.1002/jez.1402320332

Hernández-Ochoa, I., García-Vargas, G., López-Carrillo, L., Rubio-Andrade, M., Morán-Martínez, J., Cebrián, M.E., Quintanilla-Vega, B., 2005. Low lead environmental exposure alters semen quality and sperm chromatin condensation in northern Mexico. Reprod. Toxicol. 20, 221–8. doi:10.1016/j.reprotox.2005.01.007

Herring, G., Eagles-Smith, C.A., Wagner, M.T., 2016. Ground squirrel shooting and potential lead exposure in breeding avian scavengers. PLoS One 11, 1–22. doi:10.1371/journal.pone.0167926

Herring, G., Ackerman, J.T., Herzog, M.P., 2012. Mercury exposure may suppress baseline corticosterone levels in juvenile birds. Environ. Sci. Technol. 46, 6339–6346. doi:10.1021/es300668c

Herring, G., Gawlik, D.E., 2009. Stability of avian fecal corticosterone metabolite levels in frozen avian feces. J. Wildl. Manage. 73, 1010–1013. doi:10.2193/2008-398

Hirschenhauser, K., Spreitzer, K., Lepschy, M., Kotrschal, K., Möstl, E., 2012. Excreted corticosterone metabolites differ between two galliform species, Japanese Quail and Chicken, between sexes and between urine and faecal parts of droppings. J. Ornithol. 153, 1179–1188. doi:10.1007/s10336-012-0848-9

Hoffmann, F., Kloas, W., 2016. p,p′-Dichlordiphenyldichloroethylene (p,p′-DDE) can elicit antiandrogenic and estrogenic modes of action in the amphibian *Xenopus laevis*. Physiol. Behav. 167, 172–178. doi:10.1016/j.physbeh.2016.09.012

Houston, D., Cooper, J., 1975. The digestive tract of the whiteback griffon vulture and its role in disease transmission among wild ungulates. J. Wildl. Dis. 11, 306–313.

Hunt, W., 2012. Implications of sublethal lead exposure in avian scavengers. J. Raptor Res. 46, 389–393.

Iwasa, T., Matsuzaki, T., Yano, K., Irahara, M., 2017. Gonadotropin-inhibitory hormone plays roles in stress-induced reproductive dysfunction. Front. Endocrinol. (Lausanne). 8. doi:10.3389/fendo.2017.00062

Jachowski, D.S., Washburn, B.E., Millspaugh, J.J., 2015. Revisiting the importance of accounting for seasonal and diel rhythms in fecal stress hormone studies. Wildl. Soc. Bull. 39, 738–745. doi:10.1002/wsb.592

Jankowski, M.D., Franson, J.C., Möstl, E., Porter, W.P., Hofmeister, E.K., 2010. Testing independent and interactive effects of corticosterone and synergized resmethrin on the immune response to West Nile virus in chickens. Toxicology 269, 81–8. doi:10.1016/j.tox.2010.01.010

Jankowski, M.D., Wittwer, D.J., Heisey, D.M., Franson, J.C., Hofmeister, E.K., 2009. The adrenocortical response of greater sage grouse (*Centrocercus urophasianus*) to capture, ACTH Injection, and confinement, as measured in fecal samples. Physiol. Biochem. Zool. 82, 190–201. doi:10.1086/596513.The

Johnson, C.K., Kelly, T.R., Rideout, B.A., 2013. Lead in ammunition: A persistent threat to health and conservation. Ecohealth 10, 455–464. doi:10.1007/s10393-013-0896-5

Jones, B.C., Smith, A.D., Bebus, S.E., Schoech, S.J., 2016. Two seconds is all it takes: European starlings (Sturnus vulgaris) increase levels of circulating glucocorticoids after witnessing a brief raptor attack. Horm. Behav. 78, 72–78. doi:10.1016/j.yhbeh.2015.10.017

Jones, R.B., Satterlee, D.G., Waddington, D., Cadd, G.G., 2000. Effects of repeated restraint in Japanese quail genetically selected for contrasting adrenocortical responses. Physiol. Behav. 69, 317–324. doi:10.1016/S0031-9384(00)00204-3

Jung-Testas, I., Baulieu, E.E., 1998. Steroid hormone receptors and steroid action in rat glial cells of the central and peripheral nervous system. J. Steroid Biochem. Mol. Biol. 65, 243–51.

Kaushal, D., Garg, M.L., Bansal, M.R., Bansal, M.P., 1996. Biokinetics of lead in various mouse organs using radiotracer technique. Biol. Trace Elem. Res. 53, 249–260. doi:10.1007/BF02784561

Keay, J.M., Singh, J., Gaunt, M.C., Kaur, T., 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species: a literature review. J. Zoo Wildl. Med. 37, 234–44. doi:10.1638/05-050.1

Khan, M.Z., Altmann, J., Isani, S.S., Yu, J., 2002. A matter of time: evaluating the storage of fecal samples for steroid analysis. Gen. Comp. Endocrinol. 128, 57–64.

Koford, C.B., 1953. California Condor, 2nd ed. National Audobon Society, Dover Publications, New York.

Koren, L., Nakagawa, S., Burke, T., Soma, K.K., Wynne-edwards, K.E., Geffen, E., 2012a. Non-breeding feather concentrations of testosterone, corticosterone and cortisol are associated with subsequent survival in wild house sparrows. Proc. R. Soc. Biol. Sci. 279, 1560–1566. doi:10.1098/rspb.2011.2062

Koren, L., Whiteside, D., Fahlman, A., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., Wynne-Edwards, K., 2012b. Cortisol and corticosterone independence in cortisol-dominant wildlife. Gen. Comp. Endocrinol. 177, 113–9. doi:10.1016/j.ygcen.2012.02.020

Kouwenberg, A., McKay, D.W., Fitzsimmons, M.G., Storey, A.E., 2015. Measuring corticosterone in feathers using an acetonitrile/hexane extraction and enzyme immunoassay: feather corticosterone levels of food-supplemented Atlantic Puffin chicks. J. F. Ornithol. 86, 73–83. doi:10.1111/jofo.12090

Kurle, C.M., Bakker, V.J., Copeland, H., Burnett, J., Jones Scherbinski, J., Brandt, J., Finkelstein, M.E., 2016. Terrestrial scavenging of marine mammals: cross-ecosystem contaminant transfer and potential risks to endangered California condors (*Gymnogyps californianus*). Environ. Sci. Technol. acs.est.6b01990. doi:10.1021/acs.est.6b01990

Landys, M.M., Ramenofsky, M., Wingfield, J.C., 2006. Actions of glucocorticoids at a seasonal baseline as compared to stress-related levels in the regulation of periodic life processes. Gen. Comp. Endocrinol. 148, 132–149. doi:10.1016/j.ygcen.2006.02.013

Lanphear, B.P., Rauch, S., Auinger, P., Allen, R.W., Hornung, R.W., 2018. Low-level lead exposure and mortality in US adults: a population-based cohort study. Lancet Public Heal. 2667, 1–8. doi:10.1016/S2468-2667(18)30025-2

Lèche, A., Busso, J.M., Navarro, J.L., Hansen, C., Marin, R.H., Martella, M.B., 2011. Non-invasive monitoring of adrenocortical activity in Greater Rhea (*Rhea americana*) by measuring fecal glucocorticoid metabolites. J. Ornithol. 152, 839–847. doi:10.1007/s10336-011-0661-x

Legagneux, P., Gauthier, G., Chastel, O., Picard, G., Bêty, J., 2011. Do glucocorticoids in droppings reflect baseline level in birds captured in the wild? A case study in snow geese. Gen. Comp. Endocrinol. 172, 440–445. doi:10.1016/j.ygcen.2011.04.009

López-Jiménez, L., Blas, J., Tanferna, A., Cabezas, S., Marchant, T., Hiraldo, F., Sergio, F., 2017. Lifetime variation in feather corticosterone levels in a long-lived raptor. Oecologia 183. doi:10.1007/s00442-016-3708-0

Love, O.P., Breuner, C.W., Vézina, F., Williams, T.D., 2004. Mediation of a corticosterone-induced reproductive conflict. Horm. Behav. 46, 59–65. doi:10.1016/j.yhbeh.2004.02.001

Lucchini, R.G., Zoni, S., Guazzetti, S., Bontempi, E., Micheletti, S., Broberg, K., Parrinello, G., Smith, D.R., 2012. Inverse association of intellectual function with very low blood lead but not with manganese exposure in Italian adolescents. Environ. Res. 118, 65–71. doi:10.1016/j.envres.2012.08.003

Lukacs, P.M., Burnham, K.P., Anderson, D.R., 2010. Model selection bias and Freedman ' s paradox 117–125. doi:10.1007/s10463-009-0234-4

Madliger, C.L., Semeniuk, C.A.D., Harris, C.M., Love, O.P., 2015. Assessing baseline stress physiology as an integrator of environmental quality in a wild avian population: Implications for use as a conservation biomarker. Biol. Conserv. 192, 409–417. doi:10.1016/j.biocon.2015.10.021

Martin, L.B., 2009. Stress and immunity in wild vertebrates: timing is everything. Gen. Comp. Endocrinol. 163, 70–6. doi:10.1016/j.ygcen.2009.03.008

Martinez-Haro, M., Taggart, M.A., Green, A.J., Mateo, R., 2009. Avian digestive tract simulation to study the effect of grit geochemistry and food on Pb shot bioaccessibility. Environ. Sci. Technol. 43, 9480–6. doi:10.1021/es901960e

Mazerolle, M.J., 2017. AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c). R Packag. version 2.1-1. URL https://cran.rproject.org/package=AICcmodavg

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2–15. doi:10.1016/S0018-506X(02)00024-7

Meaney, M., Diorio, J., Francis, D., Widdowson, J., LaPlante, P., Caldji, C., Sharma, S., Seckl, J.R., Plotsky, P.M., 1996. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. Dev. Neurosci. 18, 49–72.

Meillère, A., Brischoux, F., Bustamante, P., Michaud, B., Parenteau, C., Marciau, C., Angelier, F., 2016. Corticosterone levels in relation to trace element contamination along an urbanization gradient in the common blackbird (*Turdus merula*). Sci. Total Environ. 566–567, 93–101. doi:10.1016/j.scitotenv.2016.05.014

Miller, R., Plessow, F., Rauh, M., Gröschl, M., Kirschbaum, C., 2013. Comparison of salivary cortisol as measured by different immunoassays and tandem mass spectrometry. Psychoneuroendocrinology 38, 50–57. doi:10.1016/j.psyneuen.2012.04.019

Millspaugh, J.J., Washburn, B.E., 2004. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. Gen. Comp. Endocrinol. 138, 189–99. doi:10.1016/j.ygcen.2004.07.002

Möstl, E., Rettenbacher, S., Palme, R., 2005. Measurement of corticosterone metabolites in birds' droppings: an analytical approach. Ann. N. Y. Acad. Sci. 1046, 17–34. doi:10.1196/annals.1343.004

Nakagawa, S., Möstl, E., Waas, J., 2003. Validation of an enzyme immunoassay to measure faecal glucocorticoid metabolites from Adelie penguins (*Pygoscelis adeliae*): a non-invasive tool for estimating. Polar Biol. 26, 491–493. doi:10.1007/s00300-003-0506-z

Newman, A.E.M., Hess, H., Woodworth, B.K., Norris, D.R., 2017. Time as tyrant: The minute, hour and day make a difference for corticosterone concentrations in wild nestlings. Gen. Comp. Endocrinol. 250, 80–84. doi:10.1016/j.ygcen.2017.05.022

Nilsson, P.B., Hollmén, T.E., Atkinson, S., Mashburn, K.L., Tuomi, P.A., Esler, D., Mulcahy, D.M., Rizzolo, D.J., 2008. Effects of ACTH, capture, and short term confinement on glucocorticoid concentrations in harlequin ducks (*Histrionicus histrionicus*). Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 149, 275–283. doi:10.1016/j.cbpa.2008.01.002

Oomen, A.G., Tolls, J., Sips, a J. a M., Van den Hoop, M. a G.T., 2003. Lead speciation in artificial human digestive fluid. Arch. Environ. Contam. Toxicol. 44, 107–15. doi:10.1007/s00244-002-1225-0

Orchinik, M., Moore, F.L., Rose, J.D., 1994. Mechanistic and functional studies of rapid corticosteroid actions. Ann. N. Y. Acad. Sci. 746, 101-112-114. doi:10.1111/j.1749-6632.1994.tb39219.x

Ouyang, J.Q., Quetting, M., Hau, M., 2012. Corticosterone and brood abandonment in a passerine bird. Anim. Behav. 84, 261–268. doi:10.1016/j.anbehav.2012.05.006

Owen, J.C., Nakamura, A., Coon, C.A., Martin, L.B., 2012. The effect of exogenous corticosterone on West Nile virus infection in Northern Cardinals (*Cardinalis cardinalis*). Vet. Res. 43, 34. doi:10.1186/1297-9716-43-34

Pain, D., 2009. A global update of lead poisoning in terrestrial birds from ammunition sources. Ingestion Lead from Spent Ammunit. Implic. Wildl. Humans 99–118. doi:10.4080/ilsa.2009.0108

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann. N. Y. Acad. Sci. 1040, 162–71. doi:10.1196/annals.1327.021

Pokras, M. a, Kneeland, M.R., 2008. Lead poisoning: using transdisciplinary approaches to solve an ancient problem. Ecohealth 5, 379–85. doi:10.1007/s10393-008-0177-x

Provencher, J.F., Forbes, M.R., Hennin, H.L., Love, O.P., Braune, B.M., Mallory, M.L., Gilchrist, H.G., 2016. Implications of mercury and lead concentrations on breeding physiology and phenology in an Arctic bird. Environ. Pollut. 218, 1014–1022. doi:10.1016/j.envpol.2016.08.052

R Core Development Team, 2011. R: A Language and Environment for Statistical Computing.

Raff, H., Homar, P.J., Burns, E.A., 2002. Comparison of two methods for measuring salivary cortisol. Clin Chem 48, 207–208.

Rattner, B. a, 2009. History of wildlife toxicology. Ecotoxicology 18, 773–83. doi:10.1007/s10646-009-0354-x

Rettenbacher, S., Möstl, E., Hackl, R., Ghareeb, K., Palme, R., 2004. Measurement of corticosterone metabolites in chicken droppings. Br. Poult. Sci. 45, 704–11. doi:10.1080/00071660400006156

Rideout, B.A., Stalis, I., Papendick, R., Pessier, A., Puschner, B., Finkelstein, M.E., Smith, D.R., Johnson, M., Mace, M., Stroud, R., Brandt, J., Burnett, J., Parish, C., Petterson, J., Witte, C., Stringfield, C., Orr, K., Zuba, J., Wallace, M., Grantham, J., 2012. Patterns of mortality in free-ranging California condors (*Gymnogyps californianus*). J. Wildl. Dis. 48, 95–112.

Romero, L.M., 2002. Seasonal changes in plasma glucocorticoid concentrations in free-living vertebrates. Gen. Comp. Endocrinol. 128, 1–24.

Romero, L.M., Fairhurst, G.D., 2016. Measuring corticosterone in feathers: Strengths, limitations, and suggestions for the future. Comp. Biochem. Physiol. -Part A Mol. Integr. Physiol. 202, 112–122. doi:10.1016/j.cbpa.2016.05.002

Romero, L.M., Reed, J.M., 2005. Collecting baseline corticosterone samples in the field: Is under 3 min good enough? Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 140, 73–79. doi:10.1016/j.cbpb.2004.11.004

Rossi-George, A., Virgolini, M.B., Weston, D., Cory-Slechta, D.A., 2009. Alterations in glucocorticoid negative feedback following maternal Pb, prenatal stress and the combination: a potential biological unifying mechanism for their corresponding disease profiles. Toxicol. Appl. Pharmacol. 234, 117–27. doi:10.1016/j.taap.2008.10.003

Sabolić, I., 2006. Common mechanisms in nephropathy induced by toxic metals. Nephron. Physiol. 104, p107-14. doi:10.1159/000095539

Sanders, T., Liu, Y., Buchner, V., Tchounwou, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Rev. Environ. Health 24, 15–45.

Schmidt, K.L., Soma, K.K., 2008. Cortisol and corticosterone in the songbird immune and nervous systems: local vs. systemic levels during development. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R103-10. doi:10.1152/ajpregu.00002.2008

Schoech, S.J., Ketterson, E.D., Nolan, V., Jr, V.N., 1999. Exogenous testosterone and the adrenocortical response in dark-eyed juncos. Auk 116, 64–72. doi:10.2307/4089454

Schoech, S.J., Romero, L.M., Moore, I.T., Bonier, F., 2013. Constraints, concerns and considerations about the necessity of estimating free glucocorticoid concentrations for field endocrine studies. Funct. Ecol. 27, 1100–1106. doi:10.1111/1365-2435.12142

Schoenle, L.A., Dudek, A.M., Moore, I.T., Bonier, F., 2017. Hormones and Behavior Red-winged blackbirds (Agelaius phoeniceus) with higher baseline glucocorticoids also invest less in incubation and clutch mass. Horm. Behav. 90, 1–7. doi:10.1016/j.yhbeh.2017.02.002

Shanks, N., Meaney, J., 1995. Neonatal Endotoxin Exposure Responsivity to Stress Alters the Development of the Axis : Early Illness and Later 15.

Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. Oecologia 166, 869–87. doi:10.1007/s00442-011-1943-y

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2010. Assessing stress in animal populations: Do fecal and plasma glucocorticoids tell the same story? Gen. Comp. Endocrinol. 166, 614–9. doi:10.1016/j.ygcen.2009.12.017

Snyder, N., Snyder, H., 2000. The California Condor: A Saga of Natural History & Conservation, Bird-Banding. Academic Press, San Diego. doi:10.2307/4510445

Stahn, C., Buttgereit, F., 2008. Genomic and nongenomic effects of glucocorticoids. Nature, Clin. Pract. Rheimatology 4, 525–33. doi:10.1038/ncprheum0898

Staley, A.M., Blanco, J.M., Dufty, A.M., Wildt, D.E., Monfort, S.L., 2007. Fecal steroid monitoring for assessing gonadal and adrenal activity in the golden eagle and peregrine falcon. J. Comp. Physiol. B. 177, 609–22. doi:10.1007/s00360-007-0159-2

Tartu, S., Angelier, F., Bustnes, J.O., Moe, B., Hanssen, S.A., Herzke, D., Gabrielsen, G.W., Verboven, N., Verreault, J., Labadie, P., Budzinski, H., Wingfield, J.C., Chastel, O., 2015a. Polychlorinated biphenyl exposure and corticosterone levels in seven polar seabird species. Environ. Pollut. 197, 173–180. doi:10.1016/j.envpol.2014.12.007

Tartu, S., Angelier, F., Herzke, D., Moe, B., Bech, C., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2014. The stress of being contaminated: Adrenocortical function and reproduction in relation to persistent organic pollutants in female black legged kittiwakes. Sci. Total Environ. 476–477, 553–560. doi:10.1016/j.scitotenv.2014.01.060

Tartu, S., Angelier, F., Wingfield, J.C., Bustamante, P., Labadie, P., Budzinski, H., Weimerskirch, H., Bustnes, J.O., Chastel, O., 2015b. Corticosterone, prolactin and egg neglect behavior in relation to mercury and legacy POPs in a long-lived Antarctic bird. Sci. Total Environ. 505, 180–188. doi:10.1016/j.scitotenv.2014.10.008

Tartu, S., Lendvai, Á.Z., Blévin, P., Herzke, D., Bustamante, P., Moe, B., Gabrielsen, G.W., Bustnes, J.O., Chastel, O., 2015. Increased adrenal responsiveness and delayed hatching date in relation to polychlorinated biphenyl exposure in Arctic-breeding black-legged kittiwakes (*Rissa tridactyla*). Gen. Comp. Endocrinol. 219, 165–172. doi:10.1016/j.ygcen.2014.12.018

Telisman, S., Colak, B., Pizent, A., Jurasović, J., Cvitković, P., 2007. Reproductive toxicity of low-level lead exposure in men. Environ. Res. 105, 256–66. doi:10.1016/j.envres.2007.05.011

Tempel, D., Gutiérrez, R., 2004. Factors related to fecal corticosterone levels in California spotted owls: implications for assessing chronic stress. Conserv. Biol. 18, 538–547. doi:10.1111/j.1523-1739.2004.00372.x

Touma, C., Palme, R., 2005. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. Ann. N. Y. Acad. Sci. 1046, 54–74. doi:10.1196/annals.1343.006

United States Fish and Wildlife Service, 2013. California Condor 5-Year Review : Summary and Evaluation.

Virgolini, M.B., Bauter, M.R., Weston, D.D., Cory-Slechta, D.A., 2006. Permanent alterations in stress responsivity in female offspring subjected to combined maternal lead exposure and/or stress. Neurotoxicology 27, 11–21. doi:10.1016/j.neuro.2005.05.012

Virgolini, M.B., Chen, K., Weston, D.D., Bauter, M.R., Cory-Slechta, D.A., 2005. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol. Sci. 87, 469–82. doi:10.1093/toxsci/kfi269

von Hippel, F.A., Miller, P.K., Carpenter, D.O., Dillon, D., Smayda, L., Katsiadaki, I., Titus, T.A., Batzel, P., Postlethwait, J.H., Buck, C.L., 2018. Endocrine disruption and differential gene expression in sentinel fish on St. Lawrence Island, Alaska: Health implications for indigenous residents. Environ. Pollut. 234, 279–287. doi:10.1016/j.envpol.2017.11.054

Walker, B.G., Boersma, P.D., Wingfield, J.C., 2005. Field endocrinology and conservation biology. Integr. Comp. Biol. 45, 12–8. doi:10.1093/icb/45.1.12

Walker, B.G., Boersma, P.D., Wingfield, J.C., 2006. Habituation of adult magellanic penguins to human visitation as expressed through behavior and corticosterone secretion. Conserv. Biol. 20, 146–154. doi:10.1111/j.1523-1739.2005.00271.x

Warnken, T., Huber, K., Feige, K., 2016. Comparison of three different methods for the quantification of equine insulin. BMC Vet. Res. 12, 196. doi:10.1186/s12917-016-0828-z

Washburn, B.E., Morris, D.L., Millspaugh, J.J., Faaborg, J., Schulz, J.H., 2002. Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. Condor 104, 558–563.

Wasser, S.K., Hunt, K.E., Brown, J.L., Cooper, K., Crockett, C.M., Bechert, U., Millspaugh, J.J., Larson, S., Monfort, S.L., 2000. A generalized fecal glucocorticoid assay for use in a diverse array of nondomestic mammalian and avian species. Gen. Comp. Endocrinol. 120, 260–275. doi:10.1006/gcen.2000.7557

Whirledge, S., Cidlowski, J.A., 2010. Glucocorticoids, stress, and fertility. Minerva Endocrinol. 35, 109–125. doi:10.1586/eem.10.1

White, L.D., Cory-Slechta, D. a, Gilbert, M.E., Tiffany-Castiglioni, E., Zawia, N.H., Virgolini, M., Rossi-George, A., Lasley, S.M., Qian, Y.C., Basha, M.R., 2007. New and evolving concepts in the neurotoxicology of lead. Toxicol. Appl. Pharmacol. 225, 1–27. doi:10.1016/j.taap.2007.08.001

Wilcoxen, T.E., Boughton, R.K., Bridge, E.S., Rensel, M. a, Schoech, S.J., 2011. Age-related differences in baseline and stress-induced corticosterone in Florida scrub-jays. Gen. Comp. Endocrinol. 173, 461–6. doi:10.1016/j.ygcen.2011.07.007

Wingfield, J.C., Kitaysky, A.S., 2002. Endocrine responses to unpredictable environmental events: stress or anti-stress hormones? Integr. Comp. Biol. 42, 600–9. doi:10.1093/icb/42.3.600

Wingfield, J.C., Ramenofsky, M., Pottinger, T.G., 1999. Stress Physiology in Animals, 1st ed. CRC Press LLC, Boca Raton.