Mercury and lead exposure in avian scavengers from the Pacific Northwest suggest risks to California condors: Implications for reintroduction and recovery

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A B S T R A C T

Mercury (Hg) and lead (Pb) are widespread contaminants that pose risks to avian scavengers. In fact, Pb exposure is the primary factor limiting population recovery in the endangered California condor (Gymnogyps californianus) and Hg can impair avian reproduction at environmentally relevant exposures. The Pacific Northwest region of the US was historically part of the condor’s native range, and efforts are underway to expand recovery into this area. To identify potential threats to reintroduced condors we assessed foraging habitats, Hg and Pb exposure, and physiological responses in two surrogate avian scavenger species (common ravens [Corvus corax] and turkey vultures [Cathartes aura]) across the region between 2012 and 2016. Mercury exposure near the Pacific coast was 17 e 27-fold higher than in inland areas, and stable carbon and sulfur isotope ratios indicated that coastal scavengers were highly reliant on marine prey. In contrast, Pb concentrations were uniformly elevated across the region, with 18% of the birds exposed to subclinical poisoning levels. Elevated Pb concentrations were associated with lower delta-aminolevulinic acid dehydratase (δ-ALAD) activity, and in ravens there was an interactive effect between Hg and Pb on fecal corticosterone concentrations. This interaction indicated that the effects of Hg and Pb exposure on the stress axis are bidirectional, and depend on the magnitude of simultaneous exposure to the other contaminant. Our results suggest that condors released to the Pacific Northwest may be exposed to both elevated Hg and Pb, posing challenges to management of future condor populations in the Pacific Northwest. Developing a robust monitoring program for reintroduced condors and surrogate scavengers will help both better understand the drivers of exposure and predict the likelihood of impaired health. These findings provide a strong foundation for such an effort, providing resource managers with valuable information to help mitigate potential risks.

1. Introduction

Avian scavengers occupy key positions at the apex of aquatic and terrestrial food chains where they are important contributors to ecosystem nutrient recycling and waste removal (Whelan et al., 2008; DeVault et al., 2016; Peisley et al., 2017). However, this life-history strategy can also result in exposure to a wide variety of environmental contaminants, such as lead (Pb) and mercury (Hg), which pose substantial risk to individual- and population-level health (Finkelstein et al., 2012; Cortés-Avizanda et al., 2016; Kurle et al., 2016). Globally, avian scavengers are often exposed to these substances, which in some cases have resulted in considerable mortalities (e.g., anticoagulant rodenticides and avian scavengers (Ogada et al., 2011; Elliott et al., 2016), diclofenac and vultures [Green et al., 2004], Pb and California condors (Gymnogyps californianus; Finkelstein et al., 2012).

In North America, avian scavengers are frequently exposed to Pb (Craighead and Bedrosian, 2008; Golden et al., 2016) and Hg (Kurle et al., 2016; West et al., 2017) from consumption of contaminated food. Despite their prevalence, the sources and pathways differ for each contaminant, thus risk to scavengers is influenced by different contaminant-specific drivers (Haig et al., 2014; Eagles-Smith et al., 2018). For example, Hg biomagnifies through food webs and is particularly elevated in both top predators and animals foraging in...
aquatic and marine environments, where methylmercury production largely occurs (Peterson et al., 2015; Ackerman et al., 2016). In contrast, Pb does not biomagnify (Soto-Jiménez et al., 2011; Cardwell et al., 2013) and environmental exposure is mostly associated with hunting, recreational shooting (Church et al., 2006; Finkelstein et al., 2012; Legagneux et al., 2014), and to a lesser extent legacy Pb-based paint (Finkelstein et al., 2003), soil and sediment Pb, and mining and smelting activities (Henny et al., 1994; Legagneux et al., 2014). Coastal habitats along the northwestern United States provide food items for avian scavengers (e.g., harbor seal [Phoca vitulina], northern elephant seal [Mirounga angustirostris], and sea lion [Zalophus californianus]) that are known contaminant vectors (Gundersen et al., 2013; Peterson et al., 2016). Specifically, marine derived food consistently contains Hg at elevated levels, often well above toxicological thresholds for birds (Kurle et al., 2016; West et al., 2017). Thus, avian scavengers that consume marine mammal carrion are at elevated risk to Hg exposure (Kurle et al., 2016; West et al., 2017). Conversely, Pb exposure is more prevalent for avian scavengers in terrestrial habitats because hunting effort and availability of Pb-contaminated carcasses is usually higher in those environments (Bakker et al., 2017; West et al., 2017) because the primary sources of Pb include Pb-based bullet fragments left in hunted carcasses, offal, and shot varmints (Bedrosian et al., 2012; Legagneux et al., 2014, Herrington et al., 2016). As the largest member of the avian scavenger guild in North America, the California condor is particularly susceptible to contaminant exposure because of its low fecundity, small population size, spatially broad foraging ecology, and preference for large-bodied carcasses such as terrestrial game species and marine mammals (Walters et al., 2010; D’Elia and Haig, 2013). Condors are also social feeders, meaning that a single contaminated carcass can poison many individuals (Sheppard et al., 2013). Historically, the California condor’s range extended from Mexico to British Columbia, and large numbers of birds inhabited the Pacific Northwest region of the United States (Oregon, Washington; hereafter Pacific Northwest; Walters et al., 2010, D’Elia and Haig, 2013). Through a combination of contaminant exposure, poaching, and habitat loss, the California condor was nearly driven to extinction (Walters et al., 2010; D’Elia and Haig, 2013). Existing California condor populations are limited to the southern portion of their historic range, and are not currently self-sustaining. Thus, ongoing recovery efforts include captive breeding and releases, food provisioning, and Pb poisoning management (Walters et al., 2010; Kurle et al., 2016; Bakker et al., 2017).

Recently, a diverse consortium of federal, state, and nongovernmental groups entered into a Memorandum of Understanding to evaluate the potential for condor recovery to its former native range in the Pacific Northwest (Condor MOU, 2018), where some of the most expansive areas of suitable habitat for the California condor remain (D’Elia et al., 2015). Evaluating contaminant threats to California condors prior to reintroduction into new regions is both challenging and critical to successful population restoration efforts. Sampling surrogate taxa with similar foraging ecology and habitat use can provide a spatially integrated assessment of contaminant exposure that approximates the marine and terrestrial habitats that may be used by California condors at new release sites (Wiemeyer et al., 1986; Walters et al., 2010). Common ravens (Corvus corax) and turkey vultures (Cathartes aura) scavenge both marine and terrestrial carcasses, are long-lived, social, have expansive home ranges (Kirk and Mossman, 1998; Boarman and Heinrich, 1999), and frequently serve as sentinel species for condors locating food (D’Elia and Haig, 2013; West et al., 2017). To facilitate an understanding of potential risk to California condors in the Pacific Northwest, we examined Hg and Pb exposure in common ravens and turkey vultures from Oregon and Washington, contrasting coastal versus interior landscapes. We used light stable isotopes ($\delta^{13}$C and $\delta^{34}$S) to understand how differences in the foraging habitats (e.g., terrestrial to marine gradient) were linked to contaminant exposure. We also measured physiological biomarkers (fetal corticosterone [FCORT] metabolites, and the deltaaminolevulinic acid dehydratase [$\delta$-ALAD] enzyme activity) to understand the potential physiological response of surrogate avian scavengers to Hg and Pb exposure.

2. Methods

2.1. Study area and field sampling

We sampled avian scavengers across the Pacific Northwest, USA during March–August 2012–2016 (Fig. 51). Regions were defined as: 1) Coast Range, 2) Willamette Valley, and 3) Northern Basin/Blue Mountains based on the U.S. Environmental Protection Agency’s (EPA’s) ecoregion III classifications (US EPA, 2017). Coast Range sites were characterized by coastal beaches or estuaries bordered by dunes, coniferous forest stands, or livestock pasture fields; all sample sites were within 10 km of the ocean. Willamette Valley sites were dominated by grass seed and livestock pasture fields, whereas the Northern Basin/Blue Mountain sites largely consisted of a mix of sagebrush habitat interspersed with high intensity irrigated agriculture (e.g., alfalfa) and cattle ranching. In each region we captured common ravens and turkey vultures using a combination of net launchers, bow nets, walk-in traps, or through lethal collection (shotgun with non-toxic shot). To avoid sampling the same individual on more than one occasion, we wing-tagged turkey vultures and leg-banded common ravens with USGS bands. To avoid sampling migrant turkey vultures that may not be reflective of local Pb and Hg exposure, we constrained vulture sampling to the May → August breeding period when they were not migrating (Kirk and Mossman, 1998). Ravens were sampled across a wider time-frame (February–August) consistent with their breeding season, because they do not migrate (Boarman and Heinrich, 1999). We collected whole blood from each bird using 20-25-gauge sodium-heparinized needles from either the brachial vein or by cardiac puncture (lethal collections only). We transferred blood to labeled EDTA vacutainers and placed samples on ice in the field, then stored them frozen at $\sim 20 \, ^\circ\text{C}$ until analysis. We also extracted up to 2 ml of fecal material directly from the cloaca of each bird using a disposable 3 ml pipette. We transferred fecal samples to cryovials and placed them on ice and later frozen in the lab at $\sim 20 \, ^\circ\text{C}$ until corticosterone metabolite analysis was conducted. All field protocols were covered under state (Oregon: 005–12, 062-13, 049-13, 062-14, 009–142, 064-15; Washington: 13–014, 14–084, 15–093, 16–103) and Federal permits (20786, 21417, MB28361A-0, 21417) and an approved Institutional Animal Care and Use Permit (4428).

2.2. Blood mercury determination

We measured total mercury (THg) concentrations in blood because >95% of the Hg in bird blood is in the form of methylmercury (Rümmer et al., 2005). We analyzed all samples for total mercury following US EPA method 7473 (US EPA, 2000) on a Nippon MA-3000 (Nippon Instruments Corporation, Tokyo, Japan). Quality assurance measures included analysis of two certified reference materials (either fish muscle tissue [DORM-4; National Research Council of Canada, Ottawa, Canada]) or lobster hepatopancreas [TORT-3; National Research Council of Canada, Ottawa, Canada]), two system and method blanks, and two duplicates per
2.3. Blood lead determination

We used a combination of inductively coupled plasma mass spectrometry (ICPMS; Thermo Scientific X-Series II CCT, Thermo Fisher Scientific, Waltham MA or an Agilent 7500ce Agilent Technologies, Santa Clara CA) and anodic stripping voltammetry (ASV; LeadCare II, Megellan Diagnosis, North Billerica, MA USA) to determine Pb concentrations in blood. Thirty-six percent of the data generated for this study were determined via ASV and then back-calculated to ICPMS-equivalent concentrations using published model functions (Herring et al., 2018).

Prior to ICPMS analysis, we digested blood samples using concentrated nitric acid following Andersen (1996). Digests were filtered through a 45 μm polyvinylidene difluoride filter and fortified with an internal indium standard. Calibration curves were prepared in aqueous solutions using a commercial Pb standard (RicoChemical Company, Arlington TX; PbP ION-100, or Spectrapure, Alfa Aesar, Ward Hill, MA). Certified reference materials (blood; Bio-Rad Lipochek level 2 and 3, or National Institute of Standards and Technology SRM 955c), method blanks, and duplicates were used for method validation. Recoveries averaged 101.5 ± 0.4%, and analysis of sample duplicates (δ13C absolute relative % difference = 0.4 ± 0.4%, δ34S = 2.8 ± 2.1%).

2.5. Physiology

2.5.1. Delta-aminolevulinic acid dehydratase (δ-ALAD)

To determine δ-ALAD activity we used an adaptation of the European standard method (Berlin and Schaller, 1974). We added 100 μl of whole blood to 1.5 ml nanopure water in a 5 ml centrifuge tube and vortexed for 10 s, followed by 1 ml of 10 μM ALA solution (Sigma, St. Louis, MO), vortexed for 10 s, and incubated in the dark at 38 °C for 60 min. One ml of trichloroacetic acid stop solution (10%) was added to stop the reaction, and vortexed for 10 s. After centrifugation for 10 min at 2000 × g we removed two 100 μl aliquots and added them to a 96-well plate. Ehrlich’s indicator reagent (100 μl) was added to each well, and plates were read on a VER-SAMax (Molecular Devices, Sunnyvale, CA) microplate reader at 555 nm wavelength. δ-ALAD activity is presented as nmol/min/ml whole blood. Absolute relative percent difference for duplicates averaged 2.1 ± 0.9%.

2.5.2. Fecal corticosterone metabolites

Fecal samples were freeze-dried to a constant mass and homogenized in the laboratory and extracted three times using the following process. A dry aliquot (~0.25 g) was mixed with 5 ml of 95% ethanol and vortexed for 30 min. After centrifugation (15 min, 2500 × g), the supernatant was transferred to a new vial. This process was repeated two more times, then the cumulative extractions were allowed to evaporate under nitrogen gas. Corticosterone metabolites were resuspended in diluted extraction buffer and measured using the DetectX® Corticosterone Enzyme Immunoassay Kit following the manufacturer’s instructions (Assay Design, Inc., Ann Arbor, MI). Absolute relative percent difference for duplicates averaged 5.2 ± 2.4%. Enzyme immunoassay kits were validated using serial dilutions and assessing parallelism with corticosterone standards.

2.6. Statistical analyses

Hg and Pb Exposure—We used generalized linear mixed-effects models to examine the influence of species and region on Hg and Pb concentrations independently. We included a species × region interaction to test whether differences between species varied across sampling regions, and removed the interaction when results were not significant (P > 0.05). We included capture location as a random effect to avoid pseudoreplication resulting from sampling replicate birds from the same location, and nested sampling site with region.

Stable Isotopes—To assess the importance of feeding ecology on contaminant exposure we first ran linear mixed-effects models with species and region as fixed factors, and either δ15N or δ34S as the response variable. As in the model above, we included capture location as a random effect and nested sampling site with region. We were not able to include a species × region interaction to determine if there were species differences at the regional level because of the lack of isotope data for each species and region combination. Next, we examined the relationship between stable isotope ratios and Hg and Pb concentrations using linear mixed-effects models with region and species as fixed factors, and δ15N and δ34S as covariates, and either Pb or Hg as the response. We included δ13C × species and δ34S × species interactions to determine if there were species-specific relationships between either Hg or Pb and either δ13C or δ34S and removed the interactions when results were not significant. In these models we also included sample location as a random effect to avoid pseudoreplication.

batch of 40 samples. Recoveries averaged 99.7 ± 1.0% and 93.6 ± 2.4% for certified reference materials and calibration checks, respectively, and the method detection limit ranged from 0.039 to 0.05 ng. Absolute relative percent difference for duplicates averaged 5.3 ± 4.8%.
resulting from sampling replicate birds at the same location and nested sampling site with region. The relationship between Hg and Pb and either region or species were assessed in previous models and as such are not reported in the results.

3. Results and Fecal Corticosterone Metabolites—Because δ-ALAD activity generally follows an exponential decline with increasing Pb exposure (Martinez-Haro et al., 2011; Finkelstein et al., 2012), linear mixed effects models are not appropriate. Therefore, we simply plotted the relationship between δ-ALAD activity and blood Pb concentrations and examined variability in δ-ALAD activity in birds with Pb exposure above and below a subclinical toxicity benchmark of 0.2 μg/g.

To determine the specificity of our corticosterone EIA in measuring FCORT metabolites, we ran an analysis of covariance with the percentage of antibody bound between the standard curve and serial dilutions of the sample extracts as the response, source of sample concentration (EIA standards and serial-diluted raven and vulture samples) as a categorical factor, corticosterone concentrations as a covariate, and a corticosterone x source interaction to determine if the slope of the relationship differed depending on the source of the sample. Serial dilutions of common raven and turkey vulture FCORT metabolite extracts yielded displacement parallel to the EIA corticosterone standard curve (F(2,10) = 2.46, P = 0.13) demonstrating specificity for both species.

To examine the influence of species, Hg, and Pb on FCORT metabolite concentrations we used linear mixed-effects models that included species x Hg, species x Pb, and Hg x Pb interactions to determine if there were species-specific FCORT metabolite responses to either Hg or Pb, and to determine if the relationship between each contaminant and FCORT metabolites was influenced by exposure to the other contaminant. We included sample location as a random effect to avoid pseudoreplication resulting from sampling replicate birds at the same location and nested sampling site with region. Both the species x Hg (F(1,76.88) = 8.44, P = 0.005), species x Pb (F(1,80.32) = 8.25, P = 0.005) interactions were significant, indicating that the relationships between each contaminant and FCORT metabolites were different in the two species. Therefore, we ran species-specific linear mixed-effects models to examine the influence of Hg and Pb on FCORT metabolite concentrations. We included Hg x Pb interaction to determine if the relationship FCORT metabolites and Hg was influenced by Pb exposure, or if the relationship FCORT metabolites and Pb was influenced by Hg exposure. To facilitate interpretation of the interaction between Hg and Pb concentrations on FCORT metabolites, we plotted the conditional coefficients of the effect of either THg or Pb on FCORT metabolites relative to the range of either Pb or THg using package interplot (Solt and Hu, 2015) in R, version 3.4.2 (R Core Team, 2016). This approach plots the changes in the coefficient of one variable in a two-way interaction term conditional on the value of the other included variable (Solt and Hu, 2015). Across all models we natural log-transformed all THg, Pb and fecal corticosterone data to improve normality of residuals and homogenize the variance structure.

3. Results

3.1. Hg and Pb exposure

During 2012–2016 we sampled 205 birds (124 common ravens and 81 turkey vultures). Across all dates and regions, blood THg concentrations (μg/g ww) ranged from 0.0004 to 3.69 in ravens and from 0.0004 to 5.97 in vultures (Table S1). Blood Pb concentrations (μg/g ww) ranged from 0.005 to 1.52 in ravens and 0.01 to 0.78 in vultures. The geometric mean (± standard error) blood THg concentrations were 0.03 ± 0.01 μg/g ww in ravens and 0.14 ± 0.03 μg/g ww in vultures, whereas geometric mean blood Pb concentrations were 0.09 ± 0.01 μg/g ww in ravens and 0.06 ± 0.01 μg/g ww in vultures. For common ravens and turkey vultures, respectively, 28% and 41% exceeded the lowest observed effect benchmark for Hg exposure (>0.2 μg/g; Ackerman et al., 2016), 7% and 19% were at moderate risk (>1.0 μg/g; Ackerman et al., 2016), and 1% and 5% were at high risk (>3.0 μg/g; Ackerman et al., 2016), and 0% and 3% were at severe risk (>4.0 μg/g; Ackerman et al., 2016) [Fig. 1A]. For common raven Pb exposure, 63% of birds contained concentrations associated with subclinical poisoning for sensitive species (0.03–0.2 μg/g; Martinez-López et al., 2004, Finkelstein et al., 2012, Epsín et al., 2015), 29% had concentrations associated with subclinical poisoning (0.2–0.5 μg/g; Franson and Pain, 2011), 4% had concentrations indicative of being clinically poisoned (0.5–1.0 μg/g; Franson and Pain, 2011) [Fig. 1B]. Similarly, 67% of turkey vultures had exposure associated with subclinical poisoning for sensitive species (0.03–0.2 μg/g; Martinez-López et al., 2004, Finkelstein et al., 2012, Epsín et al., 2015), 5% had subclinical poisoning (0.2–0.5 μg/g; Franson and Pain, 2011), 4% were clinically poisoned (0.5–1.0 μg/g; Franson and Pain, 2011), and 1% had severe clinical poisoning (>1.0 μg/g; Franson and Pain, 2011) [Fig. 1B].

Fig. 1. Total mercury (A; THg) and lead (B; Pb) concentrations in common raven (Corvus corax) and turkey vulture (Cathartes aura) blood sampled in the Pacific Northwest (Oregon and Washington) during 2012–2016. Concentrations are model-derived least-squares means ± standard error controlling for spatial variation across the study area. Stacked bars indicate proportion of population below risk (gray; <0.20 μg/g ww), above the lowest observed effect benchmark (blue; >0.2–1.0 μg/g ww), moderate risk (yellow; >1.0–3.0 μg/g ww), high risk (orange; >3.0 μg/g ww), and severe risk (red; >4.0 μg/g ww) for Hg exposure, and background concentrations (gray; <0.03 μg/g ww), subclinical poisoning for sensitive species (blue; <0.03 – <0.2 μg/g ww), subclinical poisoning (yellow; >0.2 – <0.5 μg/g ww), clinical poisoning (orange; 0.5–1.0 μg/g ww), and severe clinical poisoning (red; >1.0 μg/g ww) for Pb exposure. Mercury benchmarks were developed from Ackerman et al. (2016) and Pb benchmarks were developed from Martinez-López et al. (2004), Franson and Pain (2011), Finkelstein et al. (2012), and Epsín et al. (2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
3.2. Spatial and taxonomic variation in blood THg and Pb concentrations

Blood THg concentrations differed among regions ($F_{2,94.76} = 33.76$, $P < 0.0001$) and was marginally different between species ($F_{1,155.10} = 3.66$, $P = 0.06$), but there was no species × region interaction ($F_{2,136.30} = 0.00$, $P = 0.99$). When statistically accounting for regional differences, blood THg concentrations in vultures were 2-fold higher than in ravens (Fig. 1A). When accounting for the species effects, THg concentrations of birds from the Coast Range were 27- and 17-fold higher than in birds from Northern Basin/Blue Mountains ($P < 0.0001$) and Willamette Valley ($P < 0.0001$) regions, respectively (Fig. 2A). In contrast, blood Pb concentrations did not differ between species ($F_{1,184.00} = 0.35$, $P = 0.54$; Fig. 1B) or across regions ($F_{2,87.32} = 1.91$, $P = 0.15$; Fig. 2B), nor was there an interaction between species and region ($species \times region: F_{2,166.50} = 0.54$, $P = 0.59$).

3.3. Stable isotopes

Neither $\delta^{13}C$ ($F_{1,80.07} = 0.02$, $P = 0.90$) nor $\delta^{34}S$ ($F_{1,103.90} = 0.34$, $P = 0.56$) stable isotope ratios differed between species, but we did find regional differences in the isotope ratios ($\delta^{13}C$: $F_{2,56.02} = 12.42$, $P < 0.0001$; $\delta^{34}S$: $F_{2,52.00} = 38.74$, $P < 0.0001$). Both $\delta^{13}C$ and $\delta^{34}S$ ratios were more enriched in the Coast Range than in the Willamette Valley ($P < 0.0001$ for both; Fig. 3). $\delta^{34}S$ ratios also were more enriched in the Coast Range than Northern Basin/Blue Mountains ($P < 0.0001$: Fig. 3), but $\delta^{13}C$ ratios were similar between the two regions ($P = 0.34$). The $\delta^{34}S$ ratios were also more enriched in the Willamette Valley than the Northern Basin/Blue Mountains ($P = 0.04$; Fig. 3), whereas $\delta^{13}C$ ratios did not differ between those regions ($P = 0.27$; Fig. 3). After accounting for both region and species we found that blood Hg concentrations were positively correlated with both $\delta^{13}C$ ($F_{1,48.68} = 10.07$, $P = 0.003$, Fig. 4A) and $\delta^{34}S$ ratios ($F_{1,53.05} = 15.57$, $P = 0.0002$, Fig. 4B), and there were no interactions between species and either isotope ($\delta^{13}C$: $F_{1,44.94} = 1.26$, $P = 0.27$; $\delta^{34}S$: $F_{1,52.58} = 1.29$, $P = 0.26$). We also found a significant positive relationship between blood Pb concentrations and $\delta^{34}S$ ratios ($F_{1,55.94} = 4.21$, $P = 0.04$; Fig. 4D), whereas Pb concentrations were not correlated with $\delta^{13}C$ ratios ($F_{1,55.94} = 0.08$, $P = 0.78$; Fig. 4C), nor were there interactions between species and either isotope ratio ($\delta^{13}C$: $F_{1,40.25} = 2.89$, $P = 0.10$; $\delta^{34}S$: $F_{1,56.94} = 0.59$, $P = 0.44$).

3.4. $\delta$-ALAD and fecal corticosterone metabolites

Across both species, $\delta$-ALAD activity (nmol PBG/min/mL) was more variable below Pb concentrations of 0.2 μg/g ww, ranging from 0.8 to 24.0 versus 0.1 to 9.6 for those above this subclinical toxicity benchmark (Fig. 5). $\delta$-ALAD activity was also reduced (mean = $5.9 \pm 1.4$ SE) in birds with Pb concentrations greater than 0.2 μg/g, compared to birds with Pb exposure below the benchmark (mean = $9.9 \pm 0.6$ SE).

In turkey vultures, we did not detect any relationships between FCORT metabolite concentrations and blood Hg ($F_{1,35.65} = 0.41$, $P = 0.53$) or blood Pb ($F_{1,30.6} = 0.54$, $P = 0.47$) concentrations, nor was there an interaction between Pb and Hg concentrations on turkey vulture FCORT response ($F_{1,30.93} = 0.50$, $P = 0.49$). Conversely, in ravens we found a significant Hg × Pb interaction

Fig. 2. A. Total mercury (THg) and B. lead (Pb) concentrations (μg/g wet weight) in whole blood of avian scavengers (common raven (Corvus corax) and turkey vulture (Cathartes aura)) across regions in the Pacific Northwest (Oregon and Washington) during 2012–2016. Bars represent least squares mean ± standard error, accounting for species and sample location within each region.

Fig. 3. Stable isotope biplot for avian scavenger (common ravens (Corvus corax) and turkey vultures (Cathartes aura)) carbon ($\delta^{13}C$: ‰ versus Vienna Pee Dee Belemnite (VPDB)) and sulfur ($\delta^{34}S$: ‰ versus Vienna Canyon Diablo Troilite (VCDT)) ratios by sampling region in the Pacific Northwest (Oregon and Washington) during 2012–2016. Mean values are model derived least-squares means ± standard error accounting for sampling sites within each region.
effect of Pb on FCORT metabolites across a range of THg concentrations. This approach illustrates how the magnitude and direction of the relationship between each contaminant and FCORT changes depending upon the concentration of the other contaminant. Fecal CORT is negatively correlated with Hg when Pb concentrations are below 0.08 \( \mu g/g \) ww, not correlated with Hg between 0.08 and 0.52 \( \mu g/g \) ww Pb, and is positively correlated with Hg at elevated Pb concentrations (>0.52 \( \mu g/g \) ww; Fig. 7a). Similarly, FCORT is either not related or negatively correlated with Pb when Hg concentrations are below 0.02 \( \mu g/g \) ww. Above Hg concentrations of 0.02 \( \mu g/g \) ww, FCORT responses increase with increasing Pb exposure (Fig. 7b).

4. Discussion

Mercury and Pb exposure in avian scavengers across the Pacific Northwest was highly variable, ranging across five orders of magnitude. Mercury exposure profiles were below lowest observed effects benchmarks in the majority of birds, but nearly 20% of birds contained blood Hg concentrations above levels associated with physiological impairment, and exposure in 5% of turkey vultures (but no ravens) exceeded values associated with substantial reproductive impairment. (Ackerman et al., 2016). An even higher proportion of birds exceeded benchmarks for Pb toxicity, with 9% of vultures and 29% of ravens at levels associated with subclinical...
poisoning, and 4% of birds having enough Pb in their blood to elicit clinical poisoning (Franson and Pain, 2011). Additionally, there were strong regional differences in Hg exposure for both ravens and vultures, with birds from the coastal areas exhibiting 17–27-fold higher concentrations than the other regions studied in the Pacific Northwest. In fact, nearly 60% of birds sampled in the Coast Range region exceeded the lowest observed effect level for Hg of 0.2 μg/g ww, and 12% exceeded reproductive impairment benchmarks (Ackerman et al., 2016). In contrast, Pb exposure was relatively consistent across the Pacific Northwest for both species. Stable isotope ratios ($\delta^{13}C$ and $\delta^{34}S$) suggested that spatial variability in Hg exposure was strongly associated with reliance on food from different habitats. Specifically, marine-derived prey was an important food source for Coast Range birds, whereas interior birds relied almost exclusively on terrestrial-based prey, a pattern reported by others (Hobson et al., 1994; Kelly, 2000; Kurle et al., 2016). Importantly, the interaction between Hg and Pb on physiological responses suggests that current exposure profiles may elicit a complex suite of effects. These findings provide important considerations for California condor re-introduction efforts, with specific details of potential risks condors might face when released into the Pacific Northwest.

Although both Hg and Pb are broadly distributed across western landscapes (Eagles-Smith et al., 2016; Haig et al., 2014), the respective processes associated with exposure in avian scavengers differ markedly, likely driving the distinct exposure patterns across the Pacific Northwest (e.g., THg was highest along the Coast Range, Pb was similar across all regions). Mercury biomagnifies through food webs, primarily in aquatic and marine environments (see Ackerman et al., 2016) because of the requisite biogeochemical conditions that facilitate the conversion of inorganic Hg to methylmercury (MeHg; Marvin-DiPasquale et al., 2003, Hall et al., 2008). Thus, birds that consume aquatic-derived prey can be exposed to elevated Hg through food web magnification (Sundlof et al., 1994). Coastal raven and vulture $\delta^{13}C$ and $\delta^{34}S$ ratios reflected a higher reliance on marine-based prey relative to interior birds, and Hg exposure was positively correlated with reliance on coastal food resources (McGrew et al., 2014; Kurle et al., 2016). These findings suggest that condors reintroduced to the Pacific Northwest may be exposed to elevated concentrations of Hg if they forage in the coastal zones because of their common reliance on marine carrion (Burnett et al., 2013; Kurle et al., 2016). Similarly, several vultures from the Willamette Valley had more enriched $\delta^{34}S$, signifying more recent trips to the coast for foraging (McGrew et al., 2014).

In contrast to Hg exposure, Pb does not biomagnify through food webs and is directly related to its use and availability in the environment (Soto-Jiménez et al., 2011; Cardwell et al., 2013).
Therefore, Pb-exposure in avian scavengers is predominantly associated with consumption of carcasses that contain Pb ammunition fragments (Finkelstein et al., 2012; Legagneux et al., 2014), although in some cases exposure to Pb in avian scavengers has been linked to Pb-based paint (Finkelstein et al., 2012), or soil Pb (Legagneux et al., 2014). The availability of Pb-based bullet fragment sources across the landscape has emerged as an issue of widespread conservation concern; from shot varmints (Kelly and Johnson, 2011; Herring et al., 2016; McTee et al., 2017), to unrecovered big game carcasses and viscera (Hunt et al., 2006; Knott et al., 2010), to shot marine mammals (Goldstein et al., 1999; Colegrove et al., 2005). The relatively similar Pb-exposure profiles across regions in the Pacific Northwest suggest that avian scavenger risk to Pb is broadly distributed across the region. As a result, Pb poses a potential risk to reintroduced condors throughout the Pacific Northwest. Thus, coastal environments pose potential hazards from both Hg and Pb, whereas Pb is the primary contaminant hazard in interior habitats (Kelly and Johnson, 2011; Bakker et al., 2017).

Importantly, our interpretations of potential condor exposure are based on the use of surrogate species since condor reintroduction into the Pacific Northwest has not yet occurred. Thus, confidence in these interpretations should be based on the context of how appropriately they reflect actual condor exposure. Median Pb exposure in vultures sampled within the current condor range was 54% lower than in condors (Kelly and Johnson, 2011; Kelly et al., 2014), suggesting that surrogates may substantially underestimate actual condor Pb exposure. If this difference is representative for surrogates in the Pacific Northwest, then condors reintroduced into this region may experience median Pb concentrations around 0.13 μg/g, which is equivalent to condors in their current range.

We are unaware of any spatially overlapping data on Hg exposure in condors and either ravens or vultures. However, Hg concentrations in ravens and vultures sampled near the future condor release site in Northern California (near the Oregon-California border) were 31%–201% higher than in condor blood Hg concentrations from the current condor range in Central-Southern California (Kurle et al., 2016; West et al., 2017). These data suggest either that Hg availability is higher in coastal Northern California than in the current condor range, or that these surrogates overestimate Hg exposure relative to condors. Coastal marine fish THg concentrations are substantially higher along the northern California coast, than in southern California (Davis et al., 2016), supporting the idea that Hg availability in that region may be generally more elevated. However, marine fish THg concentrations along the Oregon and Washington coast are substantially lower than those from northern California (Davis et al., 2016). Thus, monitoring condor movement patterns and Hg exposure will be important for evaluating risk from Hg in the Pacific Northwest.

Impairment of the erythropoietic system, which produces red blood cells, is one of the most common symptoms of Pb exposure in birds. This occurs through impaired production of the δ-ALAD enzyme, a precursor to heme synthesis, resulting in anemia (Hoffman et al., 1985; Redig and Arent, 2008). δ-ALAD activity was lower and less variable in birds with Pb concentrations exceeding 0.2 μg/g than in birds with lower Pb exposure, suggesting some impairment the erythropoietic system. However, sensitivity to Pb exposure varies substantially among species (Haig et al., 2014) and δ-ALAD activity in ravens and vultures may be less sensitive to Pb exposure than in other avian scavengers. In fact, California condors exhibited almost no δ-ALAD activity when blood Pb concentrations exceeded 0.5 μg/g (Finkelstein et al., 2012). Using the exponential equation for the relationship between condor blood Pb concentrations and condor δ-ALAD activity (11.6 × 10^{-0.006} × blood Pb in nanograms/ml); Finkelstein et al., 2012) we estimate that condor δ-ALAD activity would be reduced by 54% on average and to a maximum of 99% across the Pb-exposure range observed in surrogate scavengers in the Pacific Northwest. Moreover, if median Pb concentrations in surrogate scavengers are approximately 54% lower than the condors they serve as indicators for, δ-ALAD activity could be further reduced. Although δ-ALAD activity can recover, it will remain depressed with continuous Pb exposure (Redig et al., 1991).

The hypothalamic–pituitary–adrenal (HPA) axis is an important component of the endocrine system that is sensitive to both Hg and Pb (Baos et al., 2006; Franceschini et al., 2009). These two contaminants have been shown to both promote and inhibit stress hormone regulation (Baos et al., 2006; Franceschini et al., 2009; Herring et al., 2012; Meillère et al., 2016; Provencher et al., 2017), suggesting that they act through complex mechanisms that are context-dependent with respect to other environmental stressors, and potential co-exposure to other contaminants. Whereas we did not detect a response between either Hg or Pb and FCORT in vultures, we detected a significant THg × Pb interaction in relation to FCORT concentrations in ravens. Raven FCORT metabolite concentrations were positively correlated with THg and Pb exposure, but only when birds were simultaneously exposed to high concentrations of the other contaminant. Conversely, raven FCORT metabolites were both stable or declined across the range of THg and Pb concentrations when exposed to moderate-to-low Pb or THg concentrations. These results suggest that both Hg and Pb exposure can have either positive or negative effects on the HPA axis, but the magnitude and direction of the effect is dependent on exposure to the other contaminant.

Baseline corticosterone concentrations are typically associated with critical physiological processes such as regulating metabolic function (e.g., glucose concentrations), anti-inflammatory and immunosuppressant activity (Munck et al., 1984; Landys et al., 2006), and reproductive function (Love et al., 2005; Angelier et al., 2009). Experimentally-increased baseline corticosterone concentrations in birds has resulted in a variety of effects, from decreased body mass and hemocrit (Vágasi et al., 2018), to reduced feather growth (Romero et al., 2005; Jenni-Eiermann et al., 2015), and decreased survival (Goutte et al., 2010). In contrast, experimentally-reduced baseline corticosterone concentrations can cause egg laying advancement in female birds and reduce nesting probability in male birds (Goutte et al., 2011). However, the overall effect is uncertain in ravens, and it is unclear how wide ranging this effect is on avian species because there are limited studies that have examined if Pb and Hg act together to influence hormones (see Meillère et al., 2016; Provencher et al., 2017). Regardless, this interaction signals a critical finding for understanding how simultaneous exposure to multiple environmental contaminants can influence hormone regulation. Avian scavengers that forage along the coast are also exposed to marine-derived persistent organic pollutants (e.g. organochlorines; Burnett et al., 2013, Kurle et al., 2016). In the Pacific Northwest, mean organochlorine concentrations in stranded marine mammals had lower concentrations than those stranded in coastal California (Gundersen et al., 2013), indicating that condors released into the Pacific Northwest may be at lower risk of organochlorine bioaccumulation. However, organochlorine compounds can impair hormone production (Brunström et al., 2003; Verreault et al., 2007) and cause eggshell thinning (Wienmeyer et al., 1993). Understanding the potential interactive effects of metals and persistent organic pollutants would be valuable to condor recovery efforts.

Restoration of the California condor to their former range in the Pacific Northwest will require both the development of suitable release sites, as well as minimized exposure to Pb, the primarily
population constraint in current release sites (Kelly et al., 2014; Bakker et al., 2017). Additionally, the propensity of the current condor population in California to utilize marine-derived food items combined with elevated concentrations of contaminants in surrogates Pacific Northwest coastal scavengers suggests that future releases in the Pacific Northwest could result in elevated exposure to Hg (and perhaps other persistent organic pollutants) that are known to affect avian health (Burnett et al., 2013; Ackerman et al., 2016; Kurle et al., 2016). Thus, a robust monitoring program that is carefully designed to assess contaminant exposure and health biomarkers in both condors and surrogate scavengers will be a valuable tool for understanding the spatial and temporal dynamics of environmental contaminant-based threats to successful reintroduction and management. The findings of this study provide a valuable foundation for guiding development of such a program, as well as information supporting management decisions surrounding release site selection and potential contaminant exposure in condors in the Pacific Northwest.

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Appendix A. Supplementary data

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References


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