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Elements in Whole Blood of Northwestern Crows (*Corvus caurinus*) in Alaska, USA: No Evidence for an Association with Beak Deformities

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ABSTRACT: A recent outbreak of beak deformities among resident birds in Alaska, US, has raised concern about environmental contamination as a possible underlying factor. We measured whole blood concentrations of 30 essential and nonessential elements to determine whether any were associated with beak deformities in Northwestern Crows (*Corvus caurinus*). We tested for differences between 1) adults with versus those without beak deformities and 2) unaffected adults versus juveniles. Crows with beak deformities had slightly higher levels of barium, molybdenum, and vanadium (all $P<0.05$), but concentrations were generally low and within the range of values reported from other apparently healthy wild birds. Concentrations of several elements, including selenium, were higher in birds without versus birds with beak deformities (all $P<0.05$), a difference that may be explained in part by compromised foraging ability associated with the deformities. Adult crows had higher concentrations of cadmium, silicon, and zinc than juveniles (all $P<0.05$), although differences were relatively small and values were similar to those from other wild birds. Our results suggest that neither selenium nor other tested elements are likely to be causing beak deformities in Alaskan crows. We also provide the first data on elemental concentrations in Northwestern Crows. Levels of selenium far exceeded those typically found in passerine birds and were similar to those in marine-associated waterfowl, suggesting that background levels should be interpreted relative to a species' environment.

Key words: Alaska, beak deformity, element, Northwestern Crow, selenium.

High rates of beak deformities have been documented in Northwestern Crows (*Corvus caurinus*; Fig. 1; Van Hemert and Handel 2010) and other resident species in Alaska (Handel et al. 2010), raising concern about environmental contamination as a possible underlying factor. Large clusters of gross abnormalities in wild birds are rare, and most have been associated with exposure to contaminants, particularly organic pollutants and selenium (Harris and Elliott 2011; Ohlendorf and Heinz 2011).

Alaska has a long history of mining and resource extraction, which can contribute to environmental accumulation of metals such as lead and mercury (Koski et al. 2008). Selenium can also be introduced into the environment from mining, smelting, and agricultural runoff, but in northern regions natural sources may be important (Fordyce 2013). Elevated concentrations of selenium and other potentially toxic elements occur in sea ducks from the Arctic and sub-Arctic (Henny et al. 1995; Heard et al. 2008; Wayland et al. 2008). Limited data exist for concentrations of trace elements in terrestrial or nearshore birds in Alaska.

Northwestern Crows occur year-round along the coastlines of southern Alaska, British Columbia, and Washington and forage on bivalves and intertidal invertebrates (Verbeek and Butler 1999). Like other corvids, they are often present near human communities and consume a variety of natural and anthropogenic foods (Verbeek and Butler 1999).

FIGURE 1. Northwestern Crow (*Corvus caurinus*) with beak deformity in Alaska, USA. Photo by Ron Horn.

TABLE 1. Concentrations (ppm wet weight) of elements in whole blood of Northwestern Crows (*Corvus caurinus*) sampled in Alaska, USA, March 2007 to April 2008. Data are presented as mean±SE, median (minimum–maximum). For elements with some samples below detection limits, the minimum is presented as number of "nondetects" (ND). Boldface denotes significant differences ($P<0.05$) between groups (juveniles vs. adults without beak deformity; adults without vs. with beak deformity).

Element[a]	Juvenile (*n*=34)	Adult without beak deformity (*n*=76)	Adult with beak deformity (*n*=18)
Al	0.076±0.016	0.064±0.004	0.070±0.007
	0.054 (0.028–0.571)	0.060 (0.030–0.253)	0.064 (0.031–0.125)
As	0.481±0.059	0.365±0.029	0.441±0.080
	0.404 (0.031–1.453)	0.304 (0.017–1.993)	0.318 (0.121–1.37)
B	0.154±0.012	0.172±0.009	0.141±0.008
	0.136 (0.047–0.306)	0.148 (0.052–0.424)	0.134 (0.089–0.211)
Ba	0.032±0.016	**0.039±0.013**	**0.078±0.037**
	0.003 (0.001–0.361)	**0.004 (0.001–0.593)**	**0.010 (0.002–0.491)**
Ca	63.7±0.9	67.4±1.8	68.1±5.7
	64.6 (52.1–78.3)	64.3 (54.5–145.1)	63.0 (55.5–163.8)
Cd	**0.0009±7E-05**	**0.0013±8E-05**	0.0011±0.0001
	0.001 (10 ND–0.002)	**0.001 (12 ND–0.004)**	0.001 (3 ND–0.002)
Co	0.002±0.0002	0.002±0.0001	0.002±0.0003
	0.002 (1 ND–0.005)	0.002 (0.001–0.006)	0.002 (0.001–0.006)
Cr	0.800±0.044	0.796±0.027	0.846±0.054
	0.811 (0.048–1.370)	0.790 (0.396–1.354)	0.914 (0.502–1.282)
Cu	0.668±0.019	0.682±0.011	0.728±0.065
	0.69 (0.43–0.827)	0.661 (0.478–0.946)	0.653 (0.477–1.723)
Fe	566±9	575±5	580±14
	574 (433–674)	568 (474–682)	574 (494–663)
Hg	0.145±0.013	0.151±0.011	0.148±0.027
	0.152 (0.013–0.332)	0.125 (0.016–0.411)	0.115 (0.024–0.475)
K	3859±116	3763±69	3877±189
	3768 (2429–5320)	3856 (2386–5613)	3968 (2555–5093)
Li	0.005±0.0003	**0.005±0.0002**	**0.004±0.0003**
	0.005 (0.002–0.012)	**0.005 (0.001–0.009)**	**0.004 (0.002–0.006)**
Mg	90.4±1.1	90.6±0.7	89.5±2.0
	89.9 (77.7–103)	90.8 (76.1–106)	91.7 (71.5–100.1)
Mn	0.021±0.001	0.028±0.0003	0.033±0.0003
	0.018 (0.013–0.039)	0.019 (0.012–0.138)	0.019 (0.015–0.218)
Mo	0.059±0.003	**0.055±0.001**	**0.066±0.005**
	0.059 (0.029–0.116)	**0.054 (0.031–0.085)**	**0.060 (0.033–0.127)**
Na	2249±37	2239±25	2285±54
	2218 (1986–3056)	2209 (1875–2969)	2244 (1957–2792)
Ni	0.004±0.0002	0.005±0.0005	0.022±0.018
	0.004 (0.002–0.009)	0.004 (0.002–0.041)	0.004 (0.003–0.325)
P	1965±22	1978±15	1999±48
	1966 (1694–2273)	1969 (1584–2311)	1987 (1523–2278)
Pb	0.040±0.010	0.076±0.020	0.027±0.005
	0.021 (0.005–0.250)	0.023 (0.006–1.100)	0.017 (0.007–0.08)
Sb	0.009±0.0007	0.009±0.0004	0.010±0.001
	0.009 (0.001–0.016)	0.009 (0.001–0.018)	0.009 (0.001–0.018)
Se	3.45±0.37	**3.62±0.22**	**2.49±0.25**
	2.93 (0.91–8.7)	**3.09 (0.91–9.29)**	**2.47 (1.10–4.432)**
Si	**22.1±0.7**	**23.3±0.5**	22.8±0.9
	20.6 (17.0–35.2)	**22.8 (16.9–42.8)**	21.6 (16.9–32.0)

TABLE 1. Continued.

Element[a]	Juvenile (n=34)	Adult without beak deformity (n=76)	Adult with beak deformity (n=18)
Sn	0.001±0.0002	0.002±0.0004	0.001±0.0001
	0.001 (5 ND–0.006)	0.001 (6 ND–0.021)	0.001 (3 ND–0.002)
Sr	0.058±0.007	0.076±0.007	0.074±0.016
	0.052 (0.009–0.218)	0.06 (0.008–0.320)	0.055 (0.016–0.312)
V	0.024±0.002	**0.024±0.001**	**0.031±0.003**
	0.024 (0.01–0.047)	**0.022 (0.01–0.057)**	**0.0295 (0.01–0.058)**
Zn	**4.65±0.06**	**4.85±0.05**	4.83±0.13
	4.67 (4.08–5.31)	**4.81 (3.99–6.11)**	4.72 (3.66–5.95)

[a] Ag = silver; Al = aluminum; As = arsenic; B = boron; Ba = barium; Be = beryllium; Ca = calcium; Cd = cadmium; Co = cobalt; Cr = chromium; Cu = copper; Fe = iron; Hg = mercury; K = potassium; Li = lithium; Mg = magnesium; Mn = manganese; Mo = molybdenum; Na = sodium; Ni = nickel; P = potassium; Pb = lead; Sb = antimony; Se = selenium; Si = silicon; Sn = tin; Sr = strontium; Tl = thaladium; V = vanadium; Zn = zinc.

The primary objective of this study was to evaluate whether selenium or other elements were associated with beak deformities in Northwestern Crows. Additionally, we provide preliminary baseline data for elemental concentrations in this species.

Between March 2007 and April 2008, we captured nonmolting Northwestern Crows during winter from six sites in southcentral and southeastern Alaska, US: Seward (60°7′N, 149°26′W), Kenai (60°33′N, 151°14′W), Homer (59°38′N, 151°32′W), Valdez (61°7′N, 146°21′W), Haines (59°14′N, 135°26′W), and Juneau (58°23′N, 134°38′W), using modified drop-net traps and bungee-loaded whoosh nets (Sutherland et al. 2004; Van Hemert and Handel 2010). After holding each bird in an individual kennel for ≤30 min, we collected up to 1.0 mL of blood from the brachial vein in royal-blue-top BD Vacutainer® Blood Collection Tubes (Becton, Dickinson and Company, Franklin Lakes, New Jersey, USA). Samples were kept cool for up to 6 h, stored in a –20 C freezer in the field, and transferred to –80 C for storage. We identified birds as juveniles (<1 yr old) or adults (≥1 yr) based on molt limits, rectrix shape, and mouth color (Pyle 1997). We determined sex molecularly from blood samples and classified birds as with or without a beak deformity based on criteria of Van Hemert and Handel (2010). Work was completed under authority of University of Alaska Fairbanks and US Geological Survey, Alaska Science Center Institutional Animal Use and Care committees.

We submitted whole blood samples from 128 Northwestern Crows to the Utah Veterinary Diagnostic Laboratory (Logan, Utah, USA) for analysis of silver, aluminum, arsenic, boron, barium, beryllium, calcium, cadmium, cobalt, chromium, copper, iron, mercury, potassium, lithium, magnesium, manganese, molybdenum, sodium, nickel, potassium, lead, antimony, selenium, silicon, tin, strontium, thaladium, vanadium, and zinc. Samples were digested (1:1) in trace mineral-grade nitric acid (Fisher Scientific, Pittsburg, Pennsylvania, USA) under heat. Digests were diluted with ultrapure water to a final nitric acid content of 5%, which provided a matrix for the analytical standards. Prepared samples were analyzed by inductively coupled plasma-mass spectrometry and results assessed against concentration curves of known standards. Standard curves and quality-control samples were analyzed every five samples. We used a quality control threshold of ±10% from known standards.

We compared wet weight total concentrations of elements for 1) adults with versus adults without beak deformities and 2) unaffected adults versus juveniles. We used nonparametric Kruskal-Wallis tests to com-

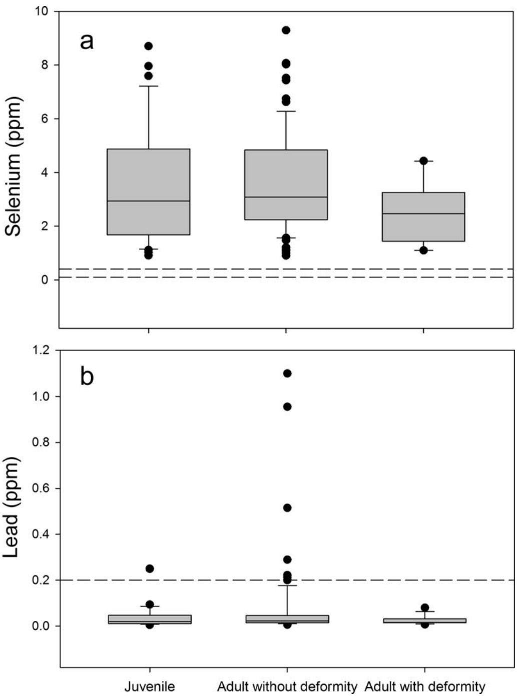

FIGURE 2. Box plots of selenium (a) and lead (b) concentrations (ppm wet weight) in whole blood of Northwestern Crows (*Corvus caurinus*) sampled in Alaska, USA, March 2007 to April 2008. Dashed lines show background levels for (a) selenium (upper and lower limit; Ohlendorf and Heinz 2011) and (b) lead (Franson and Pain 2001) in birds. Reference selenium levels are those estimated for terrestrial bird species. Seven of the eight birds with blood lead concentrations above background levels were sampled in Haines, Alaska (note that symbols for two adult values overlap).

pare groups because of small sample sizes and nonnormal distributions. For cadmium, cobalt, and tin, a small proportion (0.8–19.5%) of samples had concentrations below detection limits (<0.001 ppm for all elements). For those samples, we assigned a value equal to half the detection limit.

Whole blood samples of Northwestern Crows had detectable levels of 29 elements (Table 1). All samples were below minimum detection limits for silver. Very few samples had detectable concentrations of beryllium and thaladium, so we excluded these from summaries and analysis. We excluded lithium values for three samples collected in green-top BD Vacutainer Blood Collection Tubes due to likely contamination with lithium-heparin.

Adults with beak deformities (n=18) had higher concentrations of barium (P=0.002), molybdenum (P=0.016), and vanadium (P=0.020) and lower concentrations of lithium (P=0.010) and selenium (P=0.023; Fig. 2a) than those without beak deformities (n=76; Table 1). Juveniles (n=34) had lower concentrations of cadmium (P=0.011), silicon (P=0.009), and zinc (P=0.031) than adults without beak deformities (Table 1). No other significant differences were detected between groups.

Northwestern Crows in coastal Alaska are exposed to a broad suite of elements, including some with potentially toxic effects. Our results suggest, however, that neither selenium nor any other tested element is likely responsible for elevated rates of beak deformities in this species. Although we detected higher concentrations of barium, molybdenum, and vanadium in adult crows with versus those without beak deformities, differences were small and concentrations were within the range of values found in other studies of apparently healthy birds (Wilson et al. 2004; Heard et al. 2008). At least one statistically significant difference would be expected through chance alone because of the number of elements compared simultaneously. The effects of chronic exposure to barium, molybdenum, and vanadium are not well known, but we are unaware of any reported associations with abnormal keratin growth.

In contrast to studies of wild birds in California (Ohlendorf and Heinz 2011), selenium concentrations were lower in crows with versus those without beak deformities. Although a detailed analysis of dietary patterns was outside the scope of our study, we suspect that reduced foraging ability among affected birds may partially explain this difference. Bivalves and other marine invertebrates, which comprise a major proportion of the diet of Northwestern Crows (Verbeek and Butler 1999; O'Brien et al. 2005), are effective bioaccumulators of selenium (Fordyce 2013). Few data are available for selenium concentrations in biota from south-coastal Alaska, but studies of marine invertebrates (Rudis 1996a, b) and nearshore avian predators (Heard 2008) indicate that consumption of mussels and other bivalves is a likely route of selenium exposure for intertidally feeding Northwestern Crows. Beak deformities compromise crows' handling of natural prey, including marine invertebrates, and affected birds may instead rely on anthropogenic or other alternative food sources (e.g., Van Hemert et al. 2012). Although we did not detect differences in body mass among adults with and without beak deformities (C.V.H. unpubl. data), other observed abnormalities, including higher prevalence of ectoparasites and dermatoses (Van Hemert and Handel 2010), could be indicative of nutritional stress among affected birds.

Lithium concentrations were also lower in crows with beak deformities, but all values were near detection limits and within ranges reported in sea ducks from Alaska (Heard et al. 2008). Potential sources of lithium in the nearshore marine environment are unknown, and small differences may be biologically insignificant.

Concentrations of cadmium, silicon, and zinc were higher in adults than juveniles, a pattern reported in other studies of trace elements in blood (Wayland et al. 2011). Age-related differences were small, however, and concentrations were similar to those reported from other wild birds in Alaska and other northern regions (Wilson et al. 2004; Heard et al. 2008; Wayland et al. 2008).

Selenium concentrations in crows exceeded background levels for terrestrial species (Ohlendorf and Heinz 2011) and were similar to values from marine-associated waterfowl species (Fig. 2a; Wilson et al. 2004; Heard et al. 2008; Wayland et al. 2008). To our knowledge, Northwestern Crows are the first passerine species reported with such high blood selenium levels without apparent signs of clinical toxicosis, suggesting that published reference values may not be appropriate for all avian taxa, particularly marine-associated species. Some individual crows also had notably elevated concentrations of lead and arsenic. Eight had blood lead concentrations exceeding background values ($\leq$0.2 ppm; Fig. 2b), with three of these in the range of clinical poisoning for Anseriformes and Falconiformes ($>$0.5 ppm; Franson and Pain 2011). The highest concentrations of lead occurred in birds from Haines in southeast Alaska, where historical mining activities may have contributed to the availability of metals in the nearshore marine environment (Rudis 1996a). Five birds captured near Seward on the Gulf of Alaska had arsenic concentrations $>$1.0 ppm. Arsenic can be released as a byproduct of anthropogenic activities, but it also occurs naturally. In Alaska, elevated levels of arsenic have been reported in the marine environment in relatively pristine sites with no history of mining or other industry (Meador et al. 2004).

Our results concur with a related study of environmental contaminants in Black-capped Chickadees (*Poecile atricapillus*), which determined that neither selenium nor other elements were likely responsible for the ongoing epizootic of beak deformities in Alaskan birds (Handel and Van Hemert 2015). However, organochlorine compounds were identified as possible contributing factors among chickadees, and analyses of organic pollutants in Northwestern Crows are needed to rule out environmental contaminants as a cause of beak deformities in this species.

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