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ENVIRONMENTAL CONTAMINANTS IN EXCREMENT OF IOWA'S NESTING AND WINTERING BALD EAGLES (*HALIAEETUS LEUCOCEPHALUS*)

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ABSTRACT.—Bald Eagles (*Haliaeetus leucocephalus*) were rare only a few decades ago but have undergone a spectacular recovery range-wide. While their numbers have increased, there is concern about exposure of Bald Eagles to environmental contaminants. We collected excrement from nesting and wintering Bald Eagles in Iowa to examine their exposure to several contaminants and tested for differences as a function of space, time, and breeding status. We detected aluminum, copper, manganese, and zinc at levels above the quantitation limit (QL) in most excrement samples. These elements are all essential micronutrients normally found in living organisms. Arsenic and selenium are essential micronutrients for which fewer samples had levels above the QL. We also detected non-essential elements barium, cadmium, lead, and mercury in excrement samples, although only one sample had a cadmium level above the QL and only 26% of samples had lead levels above the QL. Geometric mean contaminant levels in excrement samples collected from nesting eagles during the spring were higher than for samples collected in the winter for aluminum, barium, copper, manganese, and zinc. The only difference we detected in contaminant levels in excrement samples was in manganese (higher for nest sites along the Mississippi River) and selenium (lower for nest sites along the Mississippi River) versus nest sites not associated with the Mississippi River. We also found that non-breeding eagles had higher levels of barium and manganese than nesting eagles. Our results can serve as a baseline for comparison with future studies investigating exposure of Bald Eagles to environmental contaminants. *Received 15 October 2015. Accepted 18 May 2016.*

Key words: Bald Eagle, contaminant, excrement, *Haliaeetus leucocephalus*, Iowa, metal.

The Bald Eagle (*Haliaeetus leucocephalus*) was rare in many parts of North America as recently as a few decades ago because of declines linked to the use of dichlorodiphenyltrichloroethane (DDT) and habitat destruction (Broley 1947). Following a ban on the use of DDT in 1972, complemented by conservation and recovery efforts, the Bald Eagle has undergone a spectacular recovery across much of its range including Iowa (Shepherd 2013). However, there are concerns about exposure of Bald Eagles to environmental contaminants, particularly lead and mercury (Rutkiewicz et al. 2011, Haig et al. 2014). The ingestion of spent lead ammunition, lead fragments embedded in food items, and lead fishing tackle has been linked to morbidity and mortality in several avian species including Bald Eagles (Fisher et al. 2006, Cade 2007, Bedrosian et al. 2012). Like lead, mercury is a nonessential element. It is a toxin with documented negative effects on bird health including neurological and reproductive impairment (Eisler 1987, Rutkiewicz et al. 2011). In an assessment of several bird species, Evers et al. (2005) found that piscivores had the highest mercury levels and that Bald Eagles were species at particularly high risk in aquatic systems. In addition to lead and mercury, which are considered nonessential elements, other elements are essential at low levels (e.g., aluminum, selenium, zinc) but may be toxic when present at high levels (Walker et al. 1996).

One of the challenges to characterizing the magnitude of contaminant exposure in living, free-flying Bald Eagles is obtaining samples. Blood is a commonly-used biomarker to quantify contaminant exposure in living birds. However, obtaining blood samples from free-flying eagles is logistically complex, obtaining sufficient samples for large scale studies is difficult, and capturing and drawing blood from Bald Eagles can be stressful for both the bird and the human (Richardson and Miller 1997). A potential alternative to using blood to quantify contaminant exposure is to test excrement because it is non-invasive and can be collected without physically handling birds.

Excrement has been used to measure and compare levels of contaminant exposure in other bird species including the domestic chicken (*Gallus gallus domesticus*; Clapp et al. 2012), Great (*Parus major*) and Blue (*P. caeruleus*) tits (Dauwe et al. 2000), Great Blue Heron (*Ardea herodias*; Fitzner et al. 1995), Peregrine Falcon (*Falco peregrinus*; Ek et al. 2004), Pied Flycatcher

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(*Ficedula hypoleuca*; Berglund et al. 2010), Little Blue Heron (*Egretta caerulea*; Spahn and Sherry 1999), and Mallard (*Anas platyrhynchos*; Martínez-Haro et al. 2010). Excrement contaminant levels have also been positively correlated with blood contaminant levels for lead in Bald Eagles (Reiter-Marolf et al. 2016) and numerous contaminants in Greylag Geese (*Anser anser*; Mateo et al. 2006). The objective of this study was to compare environmental contaminant exposure in Bald Eagles in Iowa as a function of space, time, and breeding status. No data about most of these contaminants in Bald Eagles across Iowa were available previously. Given the concern about exposure of Bald Eagles to environmental contaminants, it is important to have a baseline for comparison with future studies and other areas.

METHODS

Study Area.—We randomly selected 110 nests of Bald Eagles from a list of more than 200 nests classified as active by the Iowa Department of Natural Resources and the United States Fish and Wildlife Service in 2011. Nearly half of these nests were in the Upper Mississippi River National Wildlife and Fish Refuge in northeastern Iowa. The remaining nests were scattered throughout the rest of Iowa on both private and public lands. Nest sites sampled in 2012 were resampled in 2013 if they were active. Any nests that were found abandoned, destroyed, or inaccessible in 2013 were replaced with randomly chosen nest sites. We also opportunistically collected excrement deposited by wintering, non-nesting Bald Eagles in central Iowa.

Excrement Sampling of Wintering and Nesting Bald Eagles.—We examined contaminant exposure in nesting and wintering Bald Eagles using excrement samples collected from below nests to reduce sampling-associated disturbance. We wore disposable latex gloves and collected each sample with disposable plastic tools. We collected dried and fresh samples as available. After collection, samples were placed on ice and then stored frozen at –20 °C until processing and testing. Nest sites were accessed on foot where possible and by boat where necessary on the Mississippi River. We spent $\leq$30 min within 100 m of each nest. Excrement samples were a combination of dark fecal material and white urate. For each nest site visit, all samples collected were mixed together and tested as one sample to represent contaminant exposure for all occupants of the nest. For 19 nest site visits, we also tested samples individually to assess variation in contaminant levels among samples collected at the same nest.

Nest sampling was spatially and temporally stratified to enable us to examine patterns in elemental exposure in Iowa. First, nests were spatially stratified into a Mississippi River group and a non–Mississippi River group. As indicated above, the majority of Bald Eagles in Iowa nest in the Upper Mississippi River National Wildlife and Fish Refuge in northeastern Iowa, but as the population has increased an increasing number of nests are found in other parts of the state. To stratify nests into two groups, a 7-km^2 buffer was placed around all nests assuming a home range size after Gerrard et al. (1992). Any buffered nests that overlapped the Mississippi River (which forms the eastern border of the state) were included in the Mississippi River adjacent group. Any buffered nests that did not overlap the Mississippi River were placed in the non–Mississippi River group. We also stratified nests temporally by collecting samples at each nest site during winter/egg incubation (mid-Feb–late Mar) and again during spring/post-hatch (May–early Jun). Sampling of non-breeding birds at wintering roost sites occurred in January and February in central Iowa.

The sampling procedures described above were conducted with permission from the United States Fish and Wildlife Service (permit MB52842A-0) and the state of Iowa (permits SC-871 and SC-872).

Contaminant Testing.—Testing for aluminum, arsenic, barium, cadmium, copper, lead, manganese, selenium, and zinc occurred concurrently. Approximately 0.5 g of sample was weighed out and placed in a 50-mL polypropylene digestion tube. To the tube, 5 mL of concentrated nitric acid was added and heated in a hot block at ~95 °C for 15 min. After cooling to room temperature, an additional 2.5 mL of nitric acid was added and the tube was reheated until the volume was reduced to 5 mL. After cooling to room temperature, 5 mL of double distilled water and 2.5 mL of concentrated hydrochloric acid were added. Finally, the sample was diluted to 50 mL with deionized water. Inductively-Coupled Plasma Mass Spectrometry

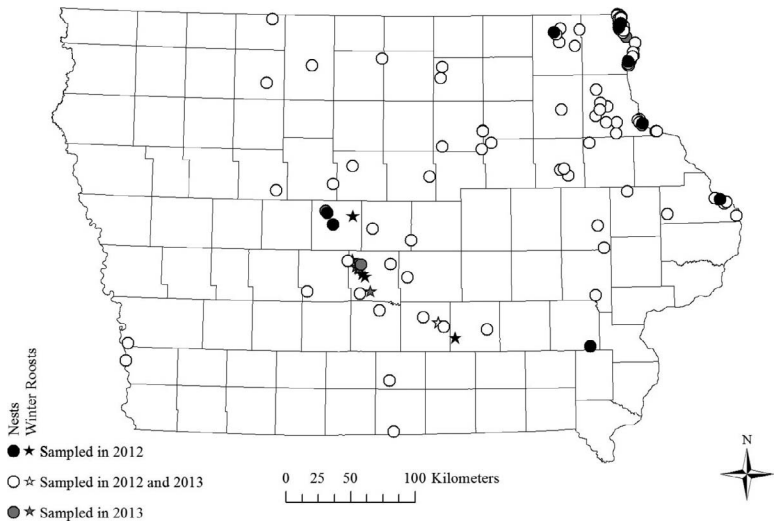

FIG. 1. Map of Iowa showing nest locations of Bald Eagles (circles) below which excrement samples were collected in 2012 ($n = 110$) and 2013 ($n = 107$) and the location of 10 winter roost sites for Bald Eagles below which excrement samples were collected in 2012 ($n = 83$) and 2013 ($n = 86$). The eastern border of Iowa is the Mississippi River.

(Agilent 7500ce, Agilent Technologies, Santa Clara, CA, USA) was used to analyze samples. Check standards, reference materials, and reagent blanks were used to evaluate accuracy. A subset of samples was tested for mercury using the same procedures described above. The quantitation limit (QL), the lowest contaminant concentration that can be reported with accuracy, for all elements was 1.0 ppm with the exception of aluminum for which the QL was 10.0 ppm and mercury for which the QL was 0.05 ppm. Element concentrations were reported in ppm dry weight.

Data Analyses.—For analyses, we assigned samples with contaminant levels below the QL a value equal to one-half the QL following Wiemeyer et al. (1989). The distributions for most contaminants were skewed. As a result, we calculated geometric rather than arithmetic means. We calculated geometric means and 95% confidence limits to compare contaminant levels a) between samples collected at nest sites during the winter and spring, b) between samples collected at nest sites from the Mississippi River and non–Mississippi River, and c) between samples collected from wintering and nesting Bald Eagles. Means whose 95% confidence limits did not overlap were considered significantly different.

RESULTS

We collected excrement at 107 nest sites between 16 February and 29 March 2012 (winter) and at 102 nest sites between 4 May and 22 May 2012 (spring) (Fig. 1). In 2013, we collected excrement at 107 nest sites between 20 February and 28 March (winter) and at 85 nest sites between 1 May and 3 June (spring). We added 16 new nest sites in 2013 to replace nests from 2012 that were no longer active. Three of the 110 nest sites each year did not yield enough excrement for testing in winter. The smaller number of nest sites sampled in spring relative to winter reflects nest sites that were no longer active (e.g., a nest failure) or nest sites along the Mississippi River that were inaccessible because of high water. In 2012, 83 excrement samples were collected from below roosts of wintering Bald Eagles between 3–10 January. In 2013, 86 excrement samples were collected from below roosts of wintering eagles between 21 January and 20 February.

To compare environmental contaminant exposure as a function of space, time, and breeding status, we tested 570 excrement samples for aluminum, arsenic, barium, cadmium, copper, lead, manganese, selenium, and zinc and 126 excrement samples (from nesting eagles only) for mercury. For six of the 10 elements, the majority

TABLE 1. Descriptive statistics of contaminant levels in excrement collected from nesting and wintering Bald Eagles in 2012 and 2013 in Iowa. The sample size for all contaminants was 570 with the exception of mercury where the sample size was 126. Results are in parts per million (ppm).

	% above quantitation limit	Geometric mean (standard deviation)	Range
Aluminum	76.1	82.3 (7.2)	<10.0–12,783
Barium	86.3	13.6 (5.2)	<1.0–230
Copper	77.0	2.9 (3.2)	<1.0–365
Lead	25.8	0.7 (2.1)	<1.0–170
Manganese	89.1	43.2 (7.2)	<1.0–3,536
Mercury	63.5	0.16 (2.68)	<0.05–0.69
Selenium	33.9	0.8 (2.1)	<1.0–21
Zinc	88.2	57.0 (8.3)	<1.0–1,853

of samples had levels above the QL (Table 1). The exceptions were arsenic, cadmium, lead, and selenium with only 3.3%, 0.2%, 25.8% and 33.9% of samples, respectively, with levels above the QL. Because few samples had levels of arsenic ($n = 19$, range 1.0–11.0 ppm) or cadmium ($n = 1$, 1.1 ppm) above the QL, these elements were not evaluated for differences as a function of space, time, or eagle breeding status.

For the 19 nest site visits where we tested samples individually rather than mixing them together, we found considerable variation in contaminant levels among samples collected from the same nest site (Supplemental Material). The magnitude of variation was highest for aluminum, manganese, and zinc. The amount of variation was lowest for elements for which most samples had levels below the QL (i.e., lead and selenium).

TABLE 2. Geometric means and 95% confidence intervals of contaminant levels in excrement collected from nesting Bald Eagles in the winter and in the spring of 2012 and 2013 in Iowa. Results are in parts per million (ppm). Contaminant concentrations that were statistically significantly different between winter and spring samples are indicated in bold.

	Winter ($n = 214$)[a]		Spring ($n = 187$)[b]	
	Mean	95% CI	Mean	95% CI
Aluminum	**40.1**	**30.4, 52.9**	**174.1**	**137.5, 220.6**
Barium	**6.8**	**5.4, 8.7**	**22.2**	**18.3, 26.8**
Copper	**2.1**	**1.8, 2.4**	**3.7**	**3.2, 4.3**
Lead	0.7	0.6, 0.8	0.8	0.7, 0.8
Manganese	**18.3**	**13.6, 24.7**	**73.0**	**57.9, 91.9**
Mercury	0.16	0.13, 0.20	0.15	0.12, 0.20
Selenium	0.8	0.7, 0.9	0.8	0.7, 0.9
Zinc	**27.2**	**19.4, 38.0**	**98.6**	**77.9, 124.9**

[a] Sample size for mercury was 88.
[b] Sample size for mercury was 38.

TABLE 3. Geometric means and 95% confidence intervals of contaminant levels in excrement collected from non–Mississippi River and Mississippi River nesting Bald Eagles in 2012 and 2013 in Iowa. Results are in parts per million (ppm). Contaminant concentrations that were statistically significantly different between samples from the Mississippi River and those not associated with the Mississippi River are indicated in bold.

	Non–Mississippi River ($n = 208$)[a]		Mississippi River ($n = 193$)[b]	
	Mean	95% CI	Mean	95% CI
Aluminum	66.8	50.8, 87.9	96.0	72.4, 127.3
Barium	11.1	8.8, 14.1	12.6	10.0, 15.9
Copper	2.5	2.1, 2.9	3.0	2.6, 3.5
Lead	0.8	0.7, 0.8	0.7	0.6, 0.8
Manganese	**24.9**	**19.2, 32.5**	**50.2**	**37.0, 68.0**
Mercury	0.17	0.13, 0.23	0.15	0.12, 0.19
Selenium	**0.9**	**0.8, 1.0**	**0.7**	**0.7, 0.8**
Zinc	39.8	29.4, 53.8	62.8	45.8, 86.1

[a] Sample size for mercury was 55.
[b] Sample size for mercury was 71.

Geometric mean levels of aluminum, barium, copper, manganese, and zinc were all higher in excrement samples collected in the spring than those collected in the winter (Table 2). No statistically significant differences between seasons were found for lead, mercury, or selenium. When comparing contaminant levels between nests from the Mississippi River versus non–Mississippi River, manganese levels were higher in samples collected at nest sites from the Mississippi River while selenium levels were higher in samples collected at nest sites not associated with the Mississippi River (Table 3). There were no statistically significant differences between these two groups of nest sites for the other contaminants. Comparing elements in nesting versus wintering Bald Eagles, barium and manganese levels were higher in wintering eagles than in nesting eagles. None of the other contaminants differed significantly between nesting and wintering eagles.

DISCUSSION

We detected aluminum, copper, manganese, and zinc at levels above the QL in most excrement

TABLE 4. Geometric means and 95% confidence intervals of contaminant levels in excrement collected from nesting and wintering Bald Eagles in 2012 and 2013 in Iowa. Results are in parts per million (ppm). Contaminant concentrations that were statistically significantly different between samples from nesting and wintering eagles are indicated in bold.

	Nesting ($n = 401$)		Wintering ($n = 169$)	
	Mean	95% CI	Mean	95% CI
Aluminum	79.5	65.3, 96.9	89.2	67.3, 118.1
Barium	**11.8**	**10.0, 13.9**	**18.9**	**15.0, 23.7**
Copper	2.7	2.4, 3.0	3.2	2.7, 3.8
Lead	0.7	0.7, 0.8	0.7	0.6, 0.8
Manganese	**34.9**	**28.5, 42.8**	**71.5**	**56.2, 91.1**
Selenium	0.8	0.7, 0.9	0.8	0.7, 0.9
Zinc	49.6	39.8, 61.7	79.6	60.9, 104.0

samples collected from nesting and wintering Bald Eagles in Iowa. These elements are all essential micronutrients normally found at low levels in living organisms, but can be toxic at high levels (Walker et al. 1996). Arsenic and selenium are essential micronutrients for which fewer samples had levels above the QL. We also detected barium, cadmium, lead, and mercury in excrement samples at levels above the QL, though we note that only one sample had a cadmium level above the QL and only 26% of samples had lead levels above the QL. None of these elements are necessary for physiological function (Walker et al. 1996). These results can serve as a baseline for comparison with future studies of contaminant exposure in Bald Eagles across Iowa as well in other locations.

The contaminant levels we observed in excrement samples collected from Bald Eagles were within the range of concentrations reported in other avian species (Ek et al. 2004, Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010, Costa et al. 2013, Martinez-Haro et al. 2013). The majority of these studies, it should be noted, focused on passerines and waterfowl. Only one other study that we are aware of has used excrement to examine contaminant exposure in a raptor (Ek et al. 2004). Average contaminant levels in our samples from Bald Eagles were relatively similar to those documented in Peregrine Falcons (Ek et al. 2004), but tended to be lower than average levels documented in most non-raptors (Morrissey et al. 2005, Tiller et al. 2005, Berglund et al. 2010, Costa et al. 2013, Martinez-Haro et al. 2013). Because of differences among bird species in physiology and life history it is difficult to draw inferences about the likely impacts of the contaminant levels we observed by comparing our results to these other studies. For most contaminants there were several instances of samples with very high levels which may indicate that some Bald Eagles in our study area were being exposed to potentially toxic levels of contaminants. This is consistent with other studies on Bald Eagles in the Midwest that have documented elevated levels of contaminants such as lead and mercury in blood and tissue samples (Rutkiewicz et al. 2011, Nam et al. 2012, Warner et al. 2014).

We found that contaminant levels in excrement samples collected from nesting eagles during the spring were higher than for samples collected in the winter for aluminum, barium, copper, manganese, and zinc. The reason for elevated levels in samples collected in the spring might be related to seasonal dietary differences, because eagles have a diverse diet that can vary both temporally and spatially (Watson et al. 1991, Hunt et al. 2002). Alternatively, the difference might be related to reproduction. In the winter, females have recently laid eggs. Copper, manganese, and zinc are known to be deposited into the egg and have been associated with eggshell quality in domestic chickens (Mabe et al. 2003). We found minimal differences in contaminant levels when we compared nests along the Mississippi River to nests not associated with the Mississippi River and when we compared nesting and non-breeding eagles.

One source of variation in our results is individual variation. Contaminant absorption, retention, and impact can vary greatly among individuals because of factors including age, sex, general health, and reproductive status (Pattee et al. 1981, Evers et al. 2005, Berglund et al. 2015). Our samples collected at nest sites during the winter were from adult birds only while samples collected in the spring would likely include contributions from hatch-year birds. We also observed considerable variation in contaminant levels among samples collected at the same nest site and then tested individually. This suggests that collecting and testing numerous samples from a nest site (either individually or mixed together) may be necessary to accurately characterize contaminant exposure at a site. We also did not

standardize the ratio of urate to feces in our samples. Our excrement samples likely varied in the proportions of each component present, which may have contributed to some of the variation we observed. Studies that use excrement to examine spatial and/or temporal patterns of contaminant exposure in birds should, if possible, standardize the ratio of urate to feces in samples. Finally, because our samples were collected non-invasively from below nests and roosts, we cannot rule out the possibility of background contamination of our samples. While we were diligent to minimize contamination in the field and we removed any obvious contamination (e.g., leaves, bark) from samples before testing, we cannot rule out the possibility of some contamination influencing our results.

Future studies of contaminant exposure in Bald Eagles could use approaches that allow the source of the contaminant to be identified and the negative impacts to be quantified. For example, Martinez-Haro et al. (2011) assessed point source lead exposure in Mallards by comparing aluminum concentrations to lead levels. Contaminant isotope ratios can also be characterized to discriminate among different sources of lead exposure (Martinez-Haro et al. 2011, Finkelstein et al. 2012, Zheng et al. 2015). Finally, adverse or toxicological effects related to contaminant exposure could be assessed by using porphyrin concentrations in excrement as a biomarker (Mateo et al. 2006, Martinez-Haro et al. 2013).

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