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LEAD, MERCURY AND OTHER METAL(LOIDS) IN BALD EAGLES (HALIAEETUS LEUCOCEPHALUS, KITPU), IN NOVA SCOTIA (MI'KMA'KI)

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ABSTRACT

Multiple tissues and feather types from seven adult male bald eagles (*Haliaeetus leucocephalus*, kitpu) were analysed for lead, mercury, arsenic, selenium, uranium and other elements via ICP-MS and direct mercury analyzer. Lead concentrations were consistently highest in bone tissue. Mercury concentrations were most elevated in organ tissues (heart, liver, kidney) but did not exceed published toxicity thresholds for large birds. Molar concentrations of selenium (Se) and mercury (Hg) for all tissues are

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correlated, and molar Hg:Se ratios were below published demethylation thresholds for waterbird mercury toxicity. Even so, it is recommended that eagle nestlings be monitored for mercury, given their higher vulnerability. Arsenic concentrations were detected in muscle, heart, kidney and liver tissues, but not in feathers, blood or bone. Uranium was measurable in all feather matrices, but not in any soft tissues or bone. Neither arsenic nor uranium were at concentrations of concern. Feather types exhibited consistent patterns, with the smaller nape feathers being below or close to detection limits for most elements. Primary, secondary and tertiary flight feathers from the seven sampled eagles were consistent in having more detectable element concentrations. To date, this dataset is among the very few to compare multiple tissues and feather types for a wide range of environmentally and biologically important elements for bald eagles.

Keywords: arsenic, blood, bone, feather, metals, mercury, muscle, organs

ACKNOWLEDGING KITPU'K IN MI'KMA'KI

It is a responsibility to work with kitpu'k (bald eagles), and we honour the messages carried in their feathers from across Mi'kma'ki, (spanning Nova Scotia, New Brunswick, Newfoundland, Quebec and Maine), the ancestral and unceded territory of the L'nu (the People). Through this work, we learn what is in the bones, tissues and blood of kitpu and understand their stories. The kitpu'k in this study were found in distress in Unama'kik, Pitukewa'kik, and Eskikewa'kik, and brought to the Cobequid Wildlife Rehabilitation Centre near Niktuipukewek, Sipekne'katik (Table 1). The lab work was carried out at Saint Mary's University in K'jipuktik, Sipekne'katik (Halifax). Given the importance of kiptu, bald eagles, in Mi'kma'ki (e.g., (Mack 2017), we included English, scientific and Mi'kmawi'simk (Hebda 2014) names for all species discussed here in that order where possible.

INTRODUCTION

Bald eagles (*Haliaeetus leucocephalus,* kitpu) across North America experience multiple environmental stressors (Burger and Gochfeld 2009; McTee *et al.* 2023; Nam *et al.* 2012; Scheuhammer *et al.* 2008a), with lead (Pb) poisoning often being among the most prominent of those concerns (Chételat *et al.* 2026; Slabe *et al.* 2022). Bald eagles and other birds across Canada exposed to spent Pb ammunition and fishing weights from scavenging carcasses left behind by hunters and anglers can accumulate significant concentrations of Pb and present

toxicological clinical signs of Pb toxicity, including those in Nova Scotia (Chételat *et al.* 2026; Fisher *et al.* 2006; Mathieu *et al.* 2020; Pain *et al.* 2009; Scheuhammer 2009). Currently, there are no provincial regulations for lead-containing fishing tackle and hunting shot, although there was a private member's bill introduced in 2023 (Bill 395, 2023). It is illegal to use lead shut, lead tackle or angling weights in federal parks and wildlife areas (CRC c. 1609, 2025; SC 1994 c.22, 1994).

In addition to Pb, there are other metal(loids) of concern to wildlife in the Atlantic region (Eleftheriou *et al.* 2017; Obeid, 2025). In particular, mercury (Hg) is of concern for freshwater ecosystems in eastern Canada (Environment and Climate Change Canada, 2017). Freshwater sportfish species and common loons loons (*Gavia immer,* gwimugw) in Nova Scotia have some of the highest tissue Hg concentrations across Canada and North America (Burgess *et al.* 2005; Depew *et al.* 2013), while piscivore fish species common in southern Nova Scotia has among the highest blood Hg concentrations for loons across North America (Burgess *et al.* 2005). This is associated with atmospheric deposition of Hg (primarily from coal burning for power generation), and the higher average acidity of Nova Scotia waterbodies, which supports increased bacterial methylation and bioavailability of Hg in ecosystems leading to vertebrates (Dennis *et al.* 2005; Depew *et al.* 2013; Little *et al.* 2015). Methyl-Hg (MeHg), the most toxic form of mercury, rapidly biomagnifies in food webs, meaning that higher-trophic animals feeding on fish and other aquatic organisms will accumulate elevated concentrations of Hg (Depew *et al.* 2013; Lavoie *et al.* 2013; Little *et al.* 2015). Elevated Hg accumulation in birds is associated with neurological and reproductive issues, flight issues, and development delays in chicks and juvenile birds (Scheuhammer *et al.* 2008a; Weech 2003).

Selenium (Se) is an essential trace element involved in many different functions of the human body (Combs 2001). Despite relatively high *total* Se concentrations in Nova Scotia soils (Jones *et al.* 2017), the naturally-acidic soils in the region generally have low *bioavailable* Se generally due to strong chemical bonds with Fe/Mn/Al oxides and other soil compounds limiting Se mobility (Dinh *et al.* 2019; Winter and Gupta 1979). Additionally, researchers have also found that Se-containing selenocysteine in tissues might be effective at counteracting Hg toxicity (Bowerman *et al.* 1994; Chapman *et al.* 2016; Eagles-Smith *et al.* 2009). This is often reported as molar ratios of Se:Hg with the assumption

that a molar excess of Se >1:1 confers protection from Hg toxicity (Eagles-Smith *et al.* 2009; Scheuhammer *et al.* 2008a).

Both arsenic (As) and uranium (U) are of geogenic origin in Nova Scotia and are found at elevated concentrations in well water. Arsenic is a class I carcinogen and human cancers (Carlin *et al.* 2016). Experimental studies have shown As contamination can also affect DNA methylation and increase oxidative stress biomarkers, both carcinogenic precursors, in passerines (Laine *et al.* 2021; Sánchez-Virosta *et al.* 2020). As occurs naturally in various geological units throughout Nova Scotia mainly as the mineral arsenopyrite (FeAsS) (Sangster and Smith 2007), and groundwater associated with those geological groups are often found to be elevated in As compared to national drinking water guidelines (Chappells *et al.* 2014). A review of As concentrations for Nova Scotia organisms have indicated that many species of invertebrates (up to 22 ppm As), freshwater fish (up to 3.5 ppm As), meadow voles (*Microtus pennsylvanicus*, up to 6.2 ppm As) and shrews (*Sorex cinereus*, up to 0.28 ppm As) can have elevated As (LeBlanc *et al.* 2019) – all of which are important prey items for bald eagles in eastern Canada (Cash *et al.* 1985; Stocek 2000).

Nova Scotia has extensive U deposits throughout the province (Drage and Kennedy 2013). While uranium mining is not presently allowed, untreated well water at some central Nova Scotia regions have been found to have elevated U (Drage and Kennedy 2013; Samolczyk *et al.* 2012), and clinical studies indicate that humans exposed to elevated U in Nova Scotia have corresponding elevated U in their hair (Moss *et al.* 1983; Zamora *et al.* 2002). There are no U concentration data for Nova Scotia birds, and globally, most studies focus on marine birds (Bond and Lavers 2011; Boryło *et al.* 2010; Strumińska-Parulska *et al.* 2013). Given the concern regarding U in Nova Scotia, we include this element in our overview.

Studies detailing contaminants in bald eagles and other raptor species for eastern Canada are quite limited especially for Nova Scotia (NS). Tissue studies to date in the Atlantic provinces have focused on legacy organic contaminants such as DDT and PCB (Dominguez *et al.* 2003; Obeid 2025; Woodley *et al.* 2004), rodenticides (Obeid 2025) as well as lead, mercury and selenium (Dominguez 1999; Obeid 2025; Scheuhammer *et al.* 2008b). Specifically for metal(loids), Chételat *et al.* (2026) analysed 60 liver samples from eight scavenging bird species in Nova Scotia as a part of a national survey of 1097 livers. They found

that bald eagle liver concentrations exceeded the Pb risk threshold of 20 ug/g dw liver the most often compared to other bird species (Chételat *et al.* 2026). Obeid (2025) as a part of a larger rodenticide survey, tested 6 livers from several raptor species for lead. They found 2 bald eagles with elevated lead (>30 ppm), one exhibiting toxicosis, although there was no note indicating where those 2 bald eagles were originally from. Scheuhammer *et al.* (2008b) reported elevated concentrations of Hg and Se in brain and liver tissue for bald eagles (N=89), mostly from British Columbia (BC) and NS. Dominguez *et al.* (1999) analysed blood samples from bald eagle nestlings and 2 adult eagles found dead (unspecified causes) from Newfoundland and found low concentrations for mercury, lead and other metal(loids). In Nova Scotia, data on lead bioaccumulation are mostly limited to intake reports at wildlife rehabilitation centres (Mathieu *et al.* 2020) and are not extensively documented in peer-reviewed literature for Nova Scotia.

The studies above typically focus on increasing number of fewer tissue samples (e.g. liver or blood) in order to assess geographical trends. Comparisons of multiple tissues and feather types for contaminant trends within the same bird is not common, but essential if modelling and risk exposure based on environmental concentrations can be conducted for bald eagles. Because bald eagles are protected species in many North American jurisdictions including Nova Scotia, many bald eagle and other Falconiformes contaminant datasets are necessarily limited to non-lethal or/and non-invasive sampling, e.g., blood, feather and egg analyses (Beyer and Meador 2011) and intake testing for rehabilitation of injured birds (Mathieu *et al.* 2020). Our objective was to establish a pilot project to survey lead, mercury and other elements in multiple tissues and feather types in NS. Ultimately, a database containing multi-tissue analysis and comparing trends of various elements across tissues and feathers within whole birds will eventually support better predictive modelling of whole-body contaminant burdens and toxicology estimates based on feather and blood monitoring datasets for Nova Scotia.

METHODS

Bald eagle sampling

The Cobequid Wildlife Rehabilitation Centre (CWRC, est. 2001) in Hilden, Nova Scotia (Niktuipukewek, Sipekne'katik) has become one of the main centres in the Maritimes for receiving and rehabilitating

injured and poisoned birds of prey. CWRC All intake raptors, loons and most waterfowl undergo capillary blood Pb testing using a LeadCare ® II Blood Lead Analyzer, a point-of-care electrochemical (anodic stripping voltammetry) diagnosis instrument, which includes adding treatment lysing reagent to the capillary tube containing a few drops of whole blood prior to analyses (Magellan Diagnostics 2021). Unfortunately, in some cases, birds arrive in a state that do not allow complete recovery due to serious injuries from accidents, electrocutions and severe Pb poisoning. In those cases, euthanasia under the oversight of a professional veterinarian is required, and the carcasses undergo necropsy.

Given the high costs of laboratory analyses and our plan to sample 11 types of tissue & feather samples for each bird, budgetary logistics strictly limited the total number of birds which we could sample. Therefore, to maintain consistency, we scanned and reviewed intake charts, including blood LeadCare© test results, x-rays (Fig 1) and necropsy notes, and WildONE logs for the available carcasses from 2017 and 2018 and stored in the CWRC freezer. Prior to selection of the eagle carcasses, we met with Mi'kmaq leaders (see acknowledgements) to discuss our approach, selection criteria and learn about the deep cultural significance of ki'tpuk in Mi'kmak'i.

Based on the guidance, we focused on adult male eagles which (Table 1) spanned a range of blood Pb including "high" Pb (>65 mg/dL, 2 birds); "moderate" blood Pb (10-30 mg/dL, 3 birds) and "low" Pb (<10 mg/dL, 2 birds), and all were males based on necropsy findings. The selected eagles were admitted to CWRC throughout the year including April (2 eagles), May (2), June (1), August (1) and November (1), but we did not see discernable trends in blood Pb with month in this sample set. The eagle carcasses processed for this study were covered under a Nova Scotia Natural Resources Scientific Permit to L.M. Campbell, signed June 6, 2018, with review and oversight by the Saint Mary's University Animal Care Committee. This project was funded by a Nova Scotia Habitat Conservation Fund grant NSHCF18_14.

Sample processing & laboratory analyses

All eagles were measured and dissected at Saint Mary's University, Halifax, Nova Scotia. Pectoral muscle, heart, liver, kidney, coagulated blood and rib-cage bones were sampled and dried at low temperatures (<60°C). Feathers, including nape, chest, and 3 types of wing feathers (primary, secondary and tertiary), were carefully removed, cleaned

Table 1 **Bald eagle intake notes and morphometrics. The Intake date (M/D/Y) and number of days in the clinic prior to death are listed. All eagles were euthanized except for BE 2018-038, which died shortly after intake. Blood Pb was measured upon intake (2 "high", >65; 4 "moderate", 10-30; and 2 "low", <10 ug/dL). All eagles were necropsied at CWRC, and confirmed as mature adult males. All bird intake data are also in WildONE (Wildlife Centre of Virginia).**

CWRC Bird ID	Intake date (N days)	Intake Blood Pb (ug/dL)	Rescue county, Mi'kma'ki location	Rescue circum-stance	Injury	X-ray	Wgt (kg)	Body length (cm)	Wing chord (cm)	Humerus length (cm)	Tarsus length (cm)	Culmen length (cm)	Bill depth (cm)	Hallux length (mm)
BE 2017-089	05/17/2017 (1)	6	Cape Breton Unama'ki	Failure to thrive / Mal-adaption	Musculoskel/ Joints/ Liga-ment Tissue /Forelimb / wing/shoulder girdle	Y	~	73	59.8	28.4	68	50	31.9	21.5
BE 2017-120	05/31/2017 (0)	6.9	Pictou Piktuk	Collision / Stationary object / Walls / windows	Nervous System/ CNS-central/brain	Y	~	74.5	65	26	94.9	54.7	36.5	20.9
BE 2018-33	04/06/2018 (1)	10	Colchester Sipekne'katik	Failure to thrive / Maladaption	Nervous System/ CNS-central/brain	Y	~	72	58	26	60	48.6	30.4	21
BE 2018-120	06/13/2018 (0)	10.7	Colchester Sipekne'katik	Behavioral Stranding	Musculoskel/ Skeleton/ Non-fracture/ Hindlimb/ pelvis	N	2.415	74.8	60	26	59.3	50.5	30.2	20

Table 1 cont'd.

CWRC Bird ID	Intake date (N days)	Intake Blood Pb (ug/dL)	Rescue county, Mi'kma'ki location	Rescue circumstance	Injury	X-ray	Wgt (kg)	Body length (cm)	Wing chord (cm)	Humerus length (cm)	Tarsus length (cm)	Culmen length (cm)	Bill depth (cm)	Hallux length (mm)
BE 2017-324	08/28/2017 (17)	22	Guysborough Eskikewa'kik	Failure to thrive / Maladaption	Nervous System/ CNS-central/brain	Y	~	70.9	55.4	25.1	62.2	57.7	35.5	24
BE 2017-391	11/24/2017 (1)	>65 (High)	Cape Breton Unama'ki	Failure to thrive / Maladaption	Nervous System/ CNS-central/brain	N	3.4	83	64.5	30	85.5	54.6	34.1	30.1
BE 2018-038	04/18/2018 (0)	>65 (High)	Colchester Sipekne'katik	Behavioral Stranding	Nervous System/ CNS-central/brain	N	3.75	77.2	64.5	26.9	93.7	57.8	36.4	23.1

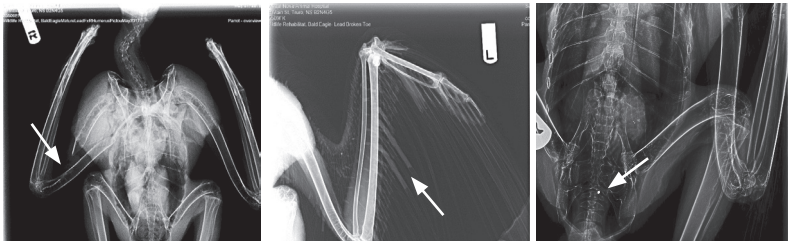


Fig 1 **Representative X-ray images of three eagles upon intake at the Cobequid Wildlife Rehabilitation Centre, Nova Scotia. The left is for BE2017-120 and shows a broken left humerus (indicated by arrow). The middle is of BE2017-324's right wing with clear images of feather camasuses. The right image is of an immature eagle (not included in this study), with bright spots in the digestive tract indicating lead particles, likely from feeding on an abandoned carcass left behind by hunters. The presence of visible Pb particles (indicated by arrow) in x-rays is unusual because visible Pb particles are not often seen in eagles upon intake. Eagles typically ingest mostly fragmented Pb from ungulate carcasses and gut piles left behind by hunters. Many of the fragments ingested from carcass remains are minuscule, and it does not take long for an eagle's powerful gizzard to grind those fragments down and entirely absorb the lead into the blood stream.**

with acetone & water, air-dried (Keute *et al.* 2024), and the barbs removed from the rachis using acetone-cleaned scissors. Tissues and cleaned feathers were pulverized into smaller sizes using acetone-cleaned mortar & pestles, homogenized to fine powder with a Retsch MM400 mixer mill and put in acid-cleaned vials. Prepared samples were shipped to the Analytical Services Unit, Queen's University, accredited by Canadian Association for Laboratory Accreditation to the standards of ISO/IEC 17025. Hg was measured on a Milestone Direct Mercury Analyzer. A total of 30 elements (Ag, Al, As, Ba, Be, Bo, Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sn, Sr, Ti, Tl, U, V and Zn) measured via ICP-MS. Calcium (Ca) concentrations of bone material exceeded the ICP-MS maximum detection limits so were measured by ICP-OES. Quality controls included blanks, prepared standards, duplicates and the NRC certified reference material TORT-2. After processing, all eagle carcasses were returned to CWRC as per Nova Scotia Department of Natural Resources protocols.

Data analyses

The primary focus of this study is on tissue concentrations & distribution of Pb, Hg and Se in bald eagles, but we also examined As and U, both of which are geogenic elements of significant environmental concern in Nova Scotia (Figs 2-6). It should be noted the sample set

is not randomized due to the nature of sampling, and therefore this set of eagles may not be representative of the bald eagle populations in Nova Scotia. Additionally, we have chose to retain the different units for ICP-MS (mass-based, µg/g) and point-of-care measurements (volume-based µg/dL for LeadCare© results) to ensure consistency with both literature and veterinary intake report conventions. We have published our full dataset of all measured elements in our Dataverse account (Campbell, 2026a), which may be of use for future wildlife and ornithology investigations.

Depending on tissue type, a number of elemental values were below the Method Detection Limit (MDL). All tissues had Hg and Fe consistently detected above MDL. Blood samples had the most results below MDL, with only 5 elements (including Mg, K, and Na) measured consistently above MDL. Bone samples had 18 elements (including) consistently above MDL. Pectoral muscle had 14 elements (including) above MDL. If more than 4 samples for each tissue type were below MDL, then we removed that tissue type from statistical analyses. An exception was blood Pb (5 blood samples below MDL) given the importance of lead contamination to birds in North America. If there were less than 3 samples per tissue type below MDL, then we adjusted for left-censoring by using the half of the LOQ value (Campbell 2026a). Eight elements (Si, Be, Bo, P, Ag, S, Tl, and Sn) were subsequently removed from the data analyses due to having no values above LOQ or having insufficient number of values above MDL for all tissue and feather types.

Lead (Pb), mercury (Hg), selenium (Se), arsenic (As) and uranium (U) separated by tissue and feather type. Box and whisker plots (Figs 2-6) were prepared based on average, standard deviation, minimum and maximum value summary statistics. The full dataset (Campbell 2026a) and the table of summary statistics (Campbell 2026b) are published in our Dataverse account.

RESULTS & DISCUSSION

Lead

Bone Pb concentrations (20±15.5 µg/g, 5.7-44.0 µg/g, Fig 2A) was most elevated, followed by liver Pb (11.7±20.1, 0.5-44.0 µg/g) and kidney Pb (5.3±7.7, 0.4-19.0 µg/g) concentrations (Fig 2). Pb concentrations from ICP-MS and point-of-care LeadCare ® II Blood Lead Analyzer

(Magellan Diagnostics, 2021) were correlated (Fig 3). Franson and Pain (2011) reviewed lead poisoning in birds across the world and define "threshold concentration" as the tissue concentration at which measurable clinical (e.g., overt symptoms) and subclinical (e.g., less visible) effects can occur. The lead tissue thresholds for each documented tissue type (blood & bone, 20 µg/g; liver, 6 µg/g; kidney, 4 µg/g) are indicated in Fig 2a (Franson and Pain, 2011). Accordingly, Pb concentrations in bone (3 eagles), kidney (2 eagles) and liver (2 eagles) tissues were above avian threshold for subclinical Pb poisoning (Franson and Pain, 2011). The 2 eagles with consistently elevated tissue Pb concentrations above the listed tissue thresholds (BE2017-391, BE2018-038) all also were in the "high Pb" category, based on intake point-of-care blood lead values (>65 µg /dL), while the third eagle (BE2017-324,) with elevated bone (but not kidney or liver) Pb concentrations was the next highest (point-of-care, 22 µg/dL). All three exhibited extreme distress upon intake, although BE2017-324 had the longest intake period (17 days) before euthanasia. Lead concentrations in feathers ranged between 0.36 to 9.4 µg/g (Fig 2B).

Recent surveys of birds of prey across Canada (liver only) and the USA (bone, liver, feather) indicated that there is a high proportion of eagles suffering from both chronic and acute lead poisoning (Chételat *et al.* 2026; Slabe *et al.* 2022). Our results above are within the reported

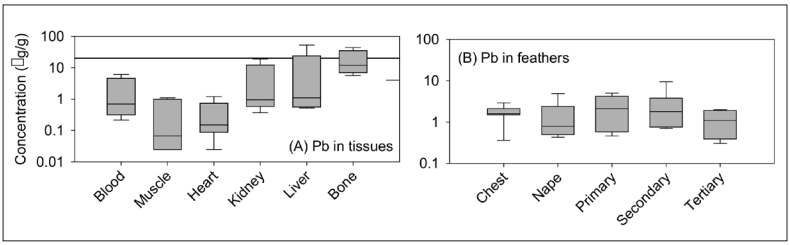


Fig 2 **Box plots showing maximum, minimum, 25th & 75th quartiles, median (solid line) & mean (dashed line) values for Pb concentrations in each type of tissue (Fig 2A) and feather (Fig 2B) for eagles processed in this study (n=7). The horizontal lines in Fig 2A indicates potential tissue thresholds postulated for bald eagle Pb poisoning symptoms from a literature review (Franson and Pain 2011). The solid line indicates the postulated blood & bone threshold for subclinical concentrations (20 mg/g) dotted line indicating upper threshold limit for subclinical concentrations in liver (6 mg/g), and the dashed line indicating upper threshold for subclinical concentrations in kidney tissues (4 mg/g). The upper limit of 100 indicates consistently severe clinical symptoms for most bird species if measured in blood. A table of summary statistics can be found at: doi.org/10.5683/SP4/C6C7BT.**

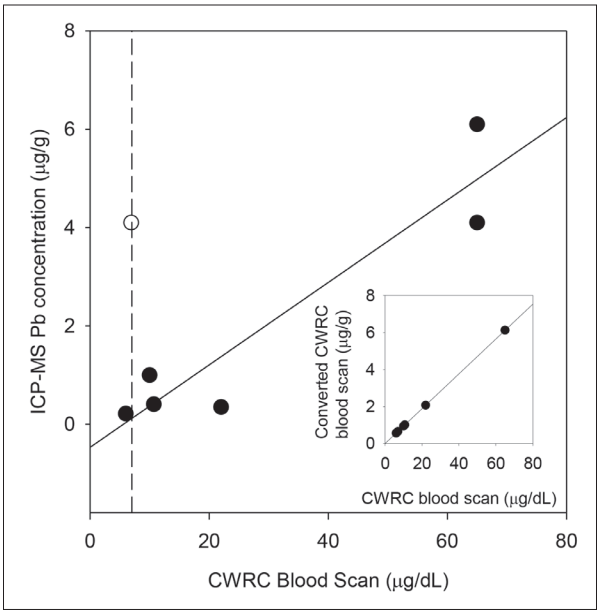


Fig 3 **Comparison of point-of-care blood scans at the Cobequid Wildlife Rehabilitation Centre (CWRC) and ICP-MS analyses of blood lead (Pb) concentrations for 7 adult bald eagles (y=-0.19+0.07(x), r^2=0.87, p=0.7). The empty circle is an outlier which was removed from the regression. The inset graph shows the relationship between converted CWRC point-of-care data using human blood density of 1.060 kg/L (Cutnell and Johnson 1998), which indicates that mg/dL and mg/g are near-analogous units for blood concentrations. The dashed line indicates the lowest published clinical Pb concentration (7 mg/dL) upon which bald eagles have demonstrated clinical symptoms. A table of summary statistics can be found at: doi.org/10.5683/SP4/C6C7BT.**

ranges for USA bald eagle femur bone (12±21 µg/g), liver (19±40 µg/g), peak feather (unspecified type of feather, 1.9±1.3 µg/g) and blood (83±271 µg/dL) from the Atlantic flyways (Slabe *et al.* 2022). In British Columbia, 37% of 294 sick, injured or dead bald eagles exhibited signs of significant Pb exposure, with 23% were sub-clinically exposed, while 14% were poisoned (Elliott *et al.* 1996). A survey of over 700 bald eagles and golden eagles (*Aquila chrysaetos*) from rehabilitation clinics across western Canada (BC, Alberta, Saskatchewan, Manitoba) found Pb concentrations (>0.2 ug/mL blood) for a significant number of birds, with high blood Pb concentrations geographically associated with areas of highest hunting density (Wayland *et al.* 2003; Wayland and Bollinger 1999). Additionally, environmental Pb exposures in bald eagles is of particular concern for hatchlings and juveniles due

to smaller sizes and development stages most vulnerable to poisoning (Hanley *et al.* 2022).

Reviewing the CWRC clinical intake logs (Table 1), we did not find correlations between immediate clinical symptoms upon intake and point-of-care blood Pb concentrations. Clinically diagnosing Pb poisoning in live bald eagles is challenging because birds can present highly variable behaviour and physiological responses among individuals. Intake logs indicate that common clinical signs of Pb toxicological responses include anisocoria (eye pupil size difference), profound weakness with a dropped head and slumped wings, laryngeal paralysis with a whistling noise, and tail feathers being stained with bright green watery feces. Tremors and seizures generally carry a grave prognosis. Based on CWRC log sheets, eagles with moderately-elevated blood Pb levels during nesting season may appear to be clinically normal, while other birds admitted to CWRC following hunting season with point-of-care blood Pb levels as low as 7 ug/dL were clinically affected. Published blood lead kinetics and the duration of Pb accumulation in birds of prey follow a consistent trend (Krone 2018), with blood Pb becoming elevated after 24 hours of ingesting fragments of spent Pb ammunition. Most blood Pb is bound to proteins and sulfhydryl compounds with a very small proportion found in serum. The half-time of blood Pb can be on the order of weeks to months since ingestion, leading to accumulation in bone, liver and kidney tissues (Franson and Pain 2011). Pb turnover in kidney and liver is about 1-3 months whereas Pb in bone is essentially immobilized with the exception of bone remodelling and eggshell production in female birds (Gangoso *et al.* 2009; Scheuhammer 1987). At the time of this study, Pb ammunition and weights are still widely available for sale for general hunting and angling in Nova Scotia, and alternative ammunition can be difficult to find. Even so, there has been public campaigns across eastern Canada to encourage hunters and anglers to switch to other non-Pb ammunition and weights such as those made with copper and bismuth (Chui, 2016).

Mercury & selenium

Hg in bald eagle tissues (Fig 4A) tended to be most elevated in kidney (9.7±8.3, 2.7-25.1 µg/g) and liver (4.1±2.5, 1.0-8.6 µg/g). The next group of Hg concentrations include muscle (1.4±0.7, 0.03-1.1 µg/g), blood (2.7±1.2, 0.9-6.1 µg/g) and heart tissue (1.5±0.6, 0.6-2.1 µg/g).

Bone (0.26±0.1, 0.2-0.4 µg/g) tissue had the lowest concentrations. Most tissues had Hg concentrations below postulated death thresholds for kidney (40 µg/g) and liver (20 µg/g) (Shore *et al.* 2011), but liver Hg was just above the postulated reproductive toxicity threshold of 2 µg/g (Shore *et al.* 2011). Feather Hg concentrations (Fig 4B) were consistent among all feather types, and all were below the feather Hg toxicity threshold (Evers *et al.* 2005). Selenium was most elevated in kidney (5.1±1.8, 3.3-8.2 µg/g) and liver (2.7±1.1, 1.7-4.9 µg/g) tissue, relative to other tissues, and was not detected in blood (Fig 4C). Feather Se concentrations ranged widely in secondary feathers (Fig 4D) but were not detected in chest or nape feathers.

The antagonistic role of selenium in demethylation (and detoxification) of Hg in wildlife, including birds, is well documented, although the actual mechanisms are still being delineated (Manceau *et al.* 2021). Molar concentrations of Se and Hg for all tissues are correlated (Fig 5). Furthermore, the sampled eagles had molar Hg:Se ratios below the demethylation threshold for Hg toxicity (8.5 µg/g) in waterbirds (Eagles-

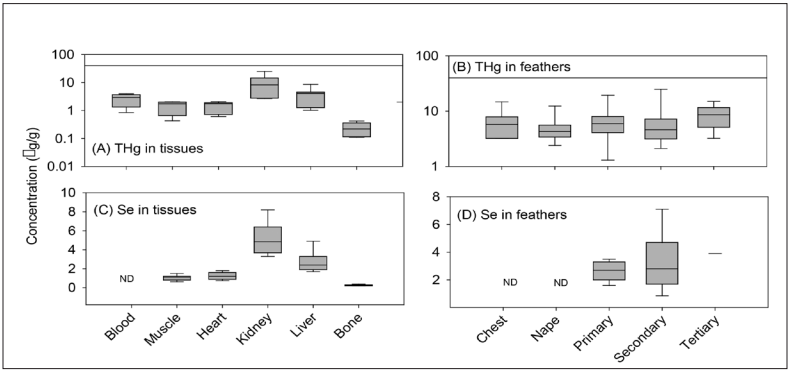


Fig 4 **Box plots showing maximum, minimum, 25th & 75th quartiles, median (solid line) & mean (dashed line) values for mercury (Hg) and selenium (Se) concentrations in each type of tissue (Fig 4A, C) and feather (Fig 4B, D) for 7 eagles processed in this study. "ND" indicates that particular element was not detected for that tissue/feather type. The horizontal lines in Fig 4 A indicates potential tissue thresholds postulated for bald eagle Hg poisoning symptoms from a literature review (Shore *et al.* 2011). The solid line indicates a postulated upper kidney Hg concentration threshold of 40 mg/g associated with death, the dotted lines indicate liver Hg concentration threshold of 20 mg/g associated with death, and the dashed line indicate the liver Hg concentration threshold for reproductive impact of 2 mg/g. The solid line in Fig 4B indicates the suggested upper Hg threshold limit of 40 mg/g for loon feathers in north-eastern North America regions (Evers *et al.* 2005). A table of summary statistics can be found at: doi.org/10.5683/SP4/C6C7BT.**

Smith *et al.* 2009), indicating that Hg concentrations may be a lesser concern for those male bald eagles. The relatively low Hg exposure and accumulation in the sampled eagles may be the result of a diverse diet incorporating marine and terrestrial prey with lower Hg concentrations than freshwater fish (Frenzel and Anthony 1989; Kozie and Anderson 1991; Stocek 2000).

Even so, nationally, Hg trends in bald eagles tend to be the most elevated in the Maritimes region (Scheuhammer *et al.* 2008a), which mirrors the trend for Hg in fish and common loons across Canada (Burgess *et al.* 2005; Depew *et al.* 2013). Bald eagles in Atlantic Canada consume a significant amount of fish, with over 60% of typical bald eagle diets in Nova Scotia and New Brunswick comprising of fish (Cash *et al.* 1985; Stocek 2000) and those in Newfoundland comprising of fish or birds (Dominguez *et al.* 2003). The increased Hg exposure through high-fish diets may still be presenting a risk to juvenile and hatchling bald eagles (Rutkiewicz *et al.* 2011; Scheuhammer *et al.* 2008a) which are more vulnerable to the risks of Hg toxicity (Scheuhammer *et al.* 2008a; Weech 2003). It is recommended that bald eagle nests in different areas of Nova Scotia be monitored via remote cameras to collect more information on prey type, feeding frequency and chick dietary composition (Houssein *et al.* 2021), and that nestlings be surveyed for blood and feather Hg trends across the region (Pittman *et al.* 2011).

Arsenic

Muscle (1.0±0.4, 0.7-1.4 µg/g), heart (0.9±0.6, 0.5-1.7 µg/g), kidney (0.9±0.3, 0.6-1.1 µg/g) and liver (1.3±0.7, 0.6-2.0 µg/g) tissues all had measurable As concentrations, while none of the feathers, blood or bone analysed in this study had measurable As concentrations above instrumentational or MDL limits. There is no published research on As accumulation in Nova Scotia bald eagles to date, although there have been published baseline studies for eagles in Alaska (feathers only), Iowa (excrement only) and a single adult eagle in Kansas (blood only) (Allen 1992; Burger and Gochfeld 2009; Reiter-Marolf *et al.* 2017).

British birds of prey species, kestrels (*Falco tinnunculus*) and sparrowhawks (*Accipiter nisus*, pi'jkwej) from a region of elevated As in southwest England had higher As in muscle (0.030-3.9 µg/g dw; 0.03-0.21µg/g dw, kestrels and sparrowhawks respectively), kidney (0.05-1.1 µg/g; 0.05-0.5 µg/g), and liver (0.04-1.4 µg/g; 0.04-0.50 µg/g) tissues

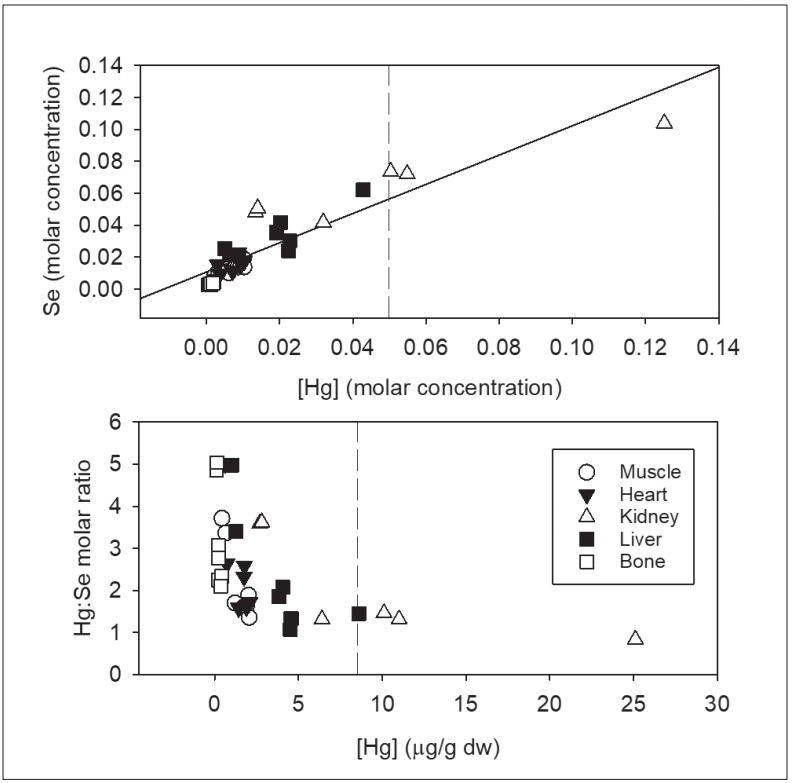


Fig 5 **Relationship between molar concentrations of mercury (Hg) and selenium (Se) in various tissues for 7 eagles processed in this study. Molar ratios of Se and Hg are significantly related ($y=-6.65+0.89$ (x), $r^2=0.82$, $p<0.05$). Feather trends did not exhibit a significant relationship between Hg & Se. The vertical dashed line is the modelled liver [Hg] demethylation threshold (8.5 mg/g dry weight liver) from a published study for waterbirds (Eagles-Smith *et al.* 2009).**

than those for same species from a reference site in south-west Scotland (Erry *et al.* 1999). A similar bird of prey investigation into osprey (*Pandion haliaetus*, ni'kmawe'su) nestlings from mining-contaminated and unimpacted regions of a large Montana (USA) river system indicated elevated blood As in nestlings from the more contaminated regions of the river system relative to those from unimpacted regions (Langner *et al.* 2012). Our data and the global literature suggest that bald eagles from Nova Scotia may be exposed to elevated arsenic and may be bioaccumulating high concentrations as a result and that further investigations are warranted.

Uranium

Our results indicate that bald eagles are accumulating total U in all feather matrices but there was no detectable U in any of the tissues sampled (Fig 6B). This is in line with human clinical literature which recommends hair samples for U monitoring (Zamora *et al.* 2002). The Nova Scotia bald eagle feather U concentrations ranged between <0.005 µg/g to 0.150 µg/g (Fig 6B), which places those birds at the lower end of the avian literature range. A survey of 234U, 238U and total U in 5 taxonomic groups of marine birds in Baltic Sea reported that fish-eating birds had the lowest U concentrations relative to those for herbivorous birds (Boryło *et al.* 2010). The authors also found that total U was consistently highest in feather matrices or in viscera for all birds, with average feather concentrations ranging from 6.3 µg/g in Eurasian coot feathers to 24 µg/g U in Great cormorant feathers. A similar study for flesh-footed shearwater feathers from the southern Pacific had a range of total U from 0.017 µg/g to 0.042 µg/g depending on their breeding region (Bond and Lavers 2011). Feeding ecology and dietary preference in eagles, in addition to the background concentrations of U may still be important factors. While accumulated U is likely not a concern for bald eagles in Nova Scotia, our results indicate that wing feathers may be a useful monitoring matrix for this element and those values are consistent with other avian studies.

Non-lethal sampling using feathers

Bald eagles will moult wing feathers consistently throughout the year (Mccollough 1989), so each feather provides a "snapshot" of contaminant exposure during the growth phase. This potentially provides a useful temporal overview of contaminant exposure during the different seasons. Documented moult patterns for bald eagles indicate that feather moult takes place from April to October, with peak moult in June & July for both wing and body feathers. Feathers are useful for assessing ages of young bald eagles up to three or four years old (Buehler 2020, McCollough 1989).

Furthermore, feather sampling is non-lethal and minimally invasive which can be done on live birds or after moulting. Non-lethal sampling approaches are especially important consideration when studying protected or vulnerable bird species (Peterson *et al.* 2019), and as such, feathers are among the most commonly sampled tissue matrix for bird contaminant studies (Gómez-Ramírez *et al.* 2014). In this limited

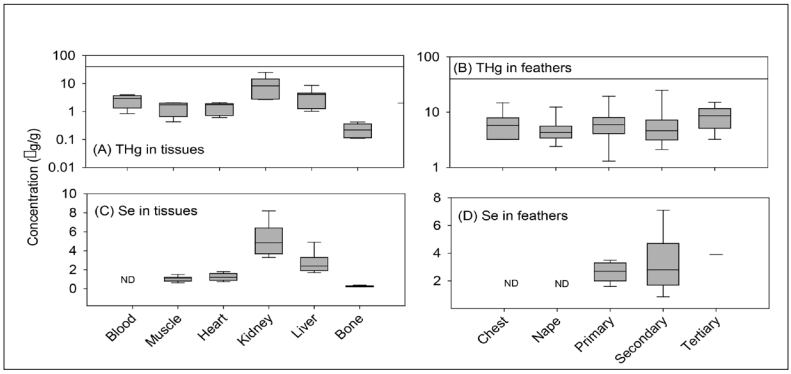


Fig 6 **Arsenic (As) concentrations in tissues and uranium (U) concentrations in feathers from eagles processed for this study (n=7). "ND" indicates that particular element was not detected for that tissue/feather type. Note that As was not detectable in any feather samples, while U was not detectable in any tissue samples. Both are naturally occurring geogenic elements of significant concern in Nova Scotia, and as a result, bald eagle tissues and feathers may represent a potential biomonitoring tool for both elements. A table of summary statistics can be found at: doi.org/10.5683/SP4/C6C7BT.**

study with 7 eagles, we did not find any correlations between feather and body tissue element concentrations for any element. This may be due to the mismatch between the timing of feather growth and the accumulation of contaminants during exposure, as well as the small number of eagles in our study significantly decreasing the power of the data analyses. Even so, feathers have been found to be a useful tool for spatiotemporal analyses (Pittman *et al.* 2011), to investigate long-term trends from historical collections (Rocque and Winker 2005) and as a "proxy" tissue for food web trophodynamics of bioaccumulating contaminants such as Hg (Einoder *et al.* 2018).

There are consistent variations in elemental concentrations across types of feathers, with the smaller nape feathers being consistently below detection limits. Chest feathers are most often used in studies as a minimally-invasive tissue matrix which does not harm the bird's ability to fly, and produces most consistent results for Hg and Pb (Burger and Gochfeld 2009; Peterson *et al.* 2019). However, in our study, several elements (Se, Co, V) were not detected in chest feathers but were detected in other feather types, including primary, secondary and tertiary wing feathers which had more numbers of detectable elemental concentrations. For a multi-elemental study, it would be recommended that the tips (~1 cm) of flight feathers be sampled to increase the range

of detected elements for analyses rather than using chest or nape feathers. In regards to live sampling of bald eagles (nestling to adults), moulting should be carefully considered when developing the feather sampling plan (Lodenius and Solonen 2013).

MANAGEMENT IMPLICATIONS

This study focused on key elements (Pb, Hg, As, Se, U) which are environmentally important in Nova Scotia. Lead trends for the seven adult eagles in this study are still of significant concern, underscoring the urgency of reducing the use of lead ammunition and weights in hunting and angling. Mercury concentrations, while of high environmental concern in Nova Scotia, appear to be below avian ecotoxicity thresholds for the sampled bald eagles based both on concentrations and molar ratios of Hg: Se. However, the Hg concentrations observed in adult eagles in this study would still be potentially concerning for hatchlings and juvenile eagles, and additional investigations would be warranted. Tissue As concentrations and feather U concentrations reveal that that bald eagles are accumulating geogenic elements of significant concern in Nova Scotia.

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IN MEMORIAM

Dr. Helene Van Doninck was a well-respected and well-known wildlife veterinarian whose work had an impact across the Maritimes region in eastern Canada. Dr. Van Doninck was a teacher, mentor, leader and innovator whose work was cut too short due to ovarian cancer.

Dr. Van Doninck has been involved with oiled wildlife preparedness and response efforts and training programs across Canada and USA since 1995. In 2001, Dr. Van Doninck co-founded the Cobequid Wildlife Rehabilitation Centre, with a bald eagle recovery centre, which has led to hundreds of eagles, other bird species and wildlife successfully returning to their habitats. Dr. Van Doninck also advocated across Canada and the United States for reduction of lead bullets and fishing weights to protect wildlife. Dr. Helene Van Doninck is missed by many, and her legacy still lives on via her very successful advocacy and leadership on behalf of all wildlife.

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