Mercury and Lead Exposure in Bald Eagles and Common Loons in the Fish River Chain of Lakes, Aroostook County, Maine





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SUBMITTED TO:

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1. EXECUTIVE SUMMARY

We initiated efforts to assess mercury (Hg) and lead (Pb) exposure in adult Common Loons and freeflying (adult and subadult) Bald Eagles in the Fish River Chain of Lakes region of northeastern Maine. This region is likely important to populations of both species from a demographic standpoint. The last statewide Bald Eagle survey indicated that Aroostook County held the fastest growing portion of Maine's resident Bald Eagle population. The region is also well-known to support large aggregations of eagles during both the winter and summer months. Common Loon surveys initiated during this study supported speculations that that this region may represent a stronghold for Common Loons in northern Maine and southeastern Canada.

We captured 13 adult Common Loons and 7 free-flying Bald Eagles (5 adults, 2 subadults) in the Study Area during a roughly 3-week field effort. We uniquely marked and collected biological samples (blood, feather) from all individuals. One inviable Common Loon egg was also collected. We analyzed all Common Loon and Bald Eagle blood, feather and egg tissues for Hg at the BRI Toxicological Laboratory in Portland, Maine. Mercury data from three Common Loons previously sampled in the Study Area were included in analyses. Whole blood samples from Common Loons and Bald Eagles were analyzed for Pb at the Michigan State University Veterinary Diagnostic Laboratory in Lansing, MI.

<u>Mercury - Loons</u>: The concentration of Hg in loon blood ranged from $0.56 - 1.67 \mu g/g$ (ww) and the mean (± SD) was $1.08 \pm 0.33 \mu g/g$ (n = 16). The mean blood Hg concentration was modestly greater in males ($1.15 \pm 0.33 \mu g/g$, n = 7) than females ($0.97 \pm 0.32 \mu g/g$, n = 7).

The concentration of Hg in loon secondary feathers ranged from $3.96 - 20.23 \ \mu g/g$ (fw). The mean (± SD) feather Hg concentration was $9.39 \pm 5.4 \ \mu g/g$ (n = 14). Male loons (mean \pm SD: 11.81 \pm 6.11, n = 8) had consistently higher feather Hg concentrations than females (6.15 \pm 1.5, n = 6) at all but one lake site. The Hg concentration in the single egg collected opportunistically was 0.34 $\mu g/g$ (ww).

We conclude that Common Loons sampled during the present study had low overall recent exposure to Hg as indicated by Hg analyses of blood and egg tissues. Analyses of feathers indicate however, that Hg is accumulating in some individuals, particularly males, over time with age. Among individuals sampled, a relatively small proportion of individuals are likely to experience adverse health effects associated with Hg (see Table 3). Since Hg bioavailability can vary substantially among lakes and even within larger lakes, additional sampling would help refine Hg risk estimates outlined in the present study.

<u>Mercury – Eagles</u>: Blood Hg concentrations in individual free-flying Bald Eagles captured in the Study Area ranged from $0.81 - 3.49 \ \mu\text{g/g}$ ww (mean \pm SD: $1.73 \pm 1.02 \ \mu\text{g/g}$, n = 7). Mercury concentrations ranged more widely in adults ($0.81 - 3.49 \ \mu\text{g/g}$, n = 5) than subadults ($1.14 - 1.25 \ \mu\text{g/g}$, n = 2), and the mean (\pm SD) Hg concentration for adults ($1.95 \pm 1.15 \ \mu\text{g/g}$ ww, n = 5) was nearly twice the mean of subadults ($1.19 \pm 0.08 \ \mu\text{g/g}$, n = 2).

The mean concentration of Hg in Bald Eagle back feathers (3 feathers averaged per individual) ranged from 8.4 μ g/g – 21.6 μ g/g (mean ± SD: 17.3 ± 5.9 μ g/g, n = 7) (Figure 8). The mean Hg concentration of feathers sampled from adult Bald Eagles (20.5 ± 2.6 μ g/g, fw, n = 5) was nearly twice the concentration found in subadults (9.3 ± 0.34 μ g/g, n = 2).

We conclude that Hg exposure in summer resident Bald Eagles our Study Area is highly variable across individuals and sites. Both blood sampling (which reflects recent dietary exposure to Hg) and feather sampling (which reflects chronic body burdens of Hg) indicated Hg exposure and potential for Hg health risks, were greater in adult than subadult eagles, which is consistent with previous studies. Mercury risks were low in subadults, but adults spanned nearly all Hg risk categories. It was notable that blood Hg concentrations exceeded 3.0 μ g/g in one individual and 3.5 μ g/g in another, since blood Hg concentrations -3.0 μ g/g are associated with a 40% reduction in reproduction (% fewer chicks fledged) in Common Loons. Our small sample size precludes making broad conclusions about Hg risk to Bald Eagles in the Study Area; preliminary sampling warrants further investigation.

Lead – Loons: Analyses of Common Loon blood samples did not indicate high Pb exposure in sampled individuals. Concentrations of Pb were below the limit of detection in all 13 samples analyzed.

We conclude that loons in our sample were not exposed to Pb via recent Pb ingestion or remobilization of previously ingested Pb at the time of sampling.

Lead – Eagles: Lead was detected in blood of 57% (4 of 7) of free-flying Bald Eagles sampled in the Study Area. Of those, Pb concentrations ranged from $3.17 - 5.47 \mu g/dl$ and averaged (± SD) $3.95 \pm 1.05 \mu g/dl$ (n = 4). Lead was below the detection limit (<100.0 to <100.2 ng/g dry weight) for the three remaining eagles sampled.

Our findings indicated that just over half of the Bald Eagles we sampled during the summer months were previously exposed to Pb; however, of those with detectable Pb in blood, concentrations were below levels typically associated with subclinical Pb poisoning (see Table 6). Small sample sizes preclude making broader conclusions about Pb exposure in our sampled population, and seasonality of exposure is an important consideration. Increased sample sizes during both the summer months (when risk of Pb exposure is lowest) and winter months (when Pb exposure risk is highest), would help clarify efforts to characterize Pb risk for the population residing in the Study Area.

Samples collected during this study provide a foundation for the creation of a baseline on Hg and Pb exposure in our two focal iconic study species. Outreach materials on the risk of Hg and Pb to wildlife and humans were also prepared as a part of this project. We make recommendations for further study given the biological importance of this region to populations of both target species.

2. INTRODUCTION

The St. John watershed in Aroostook County contains some of the most scenic and ecologically important habitats in the State of Maine. Due to its remoteness and other factors, however, this region is chronically underrepresented in many wildlife and environmental monitoring and research efforts. Two environmental issues that are perhaps studied less in northern Maine wildlife than much of the rest of the state relate to environmental contamination from two heavy metals: mercury (Hg) and lead (Pb). Although both metals occur naturally in the environment, these contaminants have notably different sources, risks and mechanisms of exposure in wildlife and humans. In this report, we focus on evaluating the exposure of two of the most well-established bioindicators – Bald Eagles (*Haliaeetus leuccocephalus*) and Common Loons (*Gavia immer*) – to Hg and Pb in northeastern Maine, U.S.

Mercury (Hg) is a toxic heavy metal that is broadly present in aquatic and terrestrial ecosystems throughout the globe. While natural sources of Hg, such as volcanoes and natural deposits are responsible for a portion of global environmental Hg pollution, anthropogenic activities (i.e., gold and mercury mining, coal-fired power plants, chlor-alkali facilities, landfills) are responsible for the majority of Hg pollution. Across much of the globe, the majority of Hg in the environment originates from anthropogenic sources of air pollution. Once deposited, sulfur-reducing bacteria and other microbes convert the inorganic and non-toxic form of Hg to a toxic organic form, methylmercury (MeHg). Exposure to MeHg via diet has a wide array of negative neurological, physiological, behavioral and reproductive impacts on wildlife at levels commonly found in Maine's freshwater ecosystems (Kamman et al. 2005, Ackerman et al. 2016). While industrial point sources have caused significant contamination in some areas of Maine such as the Penobscot River Estuary (Bodaly 2018), atmospheric deposition remains the primary source of Hg to many of Maine's lakes and rivers, particularly in remote regions. Due to prevailing wind patterns, Hg deposition is especially high in northeastern North America (Evers and Clair 2005). Once present in ecosystems, MeHg (generally referred to as Hg hereafter) bioaccumulates in organisms and biomagnifies up foodwebs. Due to their high trophic level, fish-eating birds such as Bald Eagles and Common Loons can be particularly exposed to high concentrations of Hg via their regular diet. As a result, These two species are commonly sampled to monitor spatial and temporal patterns of Hg in the environment (Bowerman et al. 2002, Evers et al. 2007), and to inform Hg risk assessments.

Bald Eagles and Common Loons in Maine are among the most Hg-contaminated populations in North America (Evers et al. 2008*a*, DeSorbo et al. 2009, 2018*c*). High levels of Hg exposure have been linked to reduced productivity in a portion of Maine's Common Loon population (Evers et al. 2008*a*), and analyses also suggest that Hg limits eagle productivity at a portion of inland sites throughout Maine (DeSorbo and Evers 2007, DeSorbo et al. 2009).

Few efforts to date have assessed Hg exposure of wildlife residing in northern Maine. Sampling in this region is of interest to researchers, however, because habitat sensitivity models suggest that habitats in this region may be well-suited to transforming atmospheric inorganic Hg inputs into the MeHg (Kamman et al. 2005, Evers et al. 2007). Prior sampling in the early 2000s suggested that Bald Eagle nestlings in the Saint John River Watershed might be exposed to higher levels of Hg than many other watersheds in the state (DeSorbo 2007, Evers et al. 2007, DeSorbo et al. 2009), and limited

Common Loon sampling (n = 3) in the late 1990s suggested Hg exposure was low (BRI, unpublished data); however, no comparable sampling has occurred in the region since.

Lead (Pb) is a highly toxic substance that has long been recognized as a threat to human and wildlife health. Birds are sensitive to elevated Pb exposure. Negative health effects of Pb range across a spectrum of potential impacts from sublethal neurological or physical impairments to acute toxicity and death (Hunt 2012). Lead poisoning is especially well-documented in Bald Eagles (Pain et al. 2009, Bedrosian et al. 2012) and Common Loons (Haig et al. 2014, Grade et al. 2017); however, the typical Pb exposure pathway differs between the two species. Ingested fishing tackle is the primary source of Pb to Common Loons and other waterbirds, while Bald Eagles are most commonly exposed to Pb through the inadvertent consumption of Pb fragments encountered while scavenging animal carcasses or gut piles discarded by hunters (Pain et al. 2009, Warner et al. 2014). Numerous studies demonstrate that Pb poisoning can negative consequences on the stability of wildlife populations. For example, Grade et al. (2017) estimated that Pb mortality in New Hampshire Common Loons reduced the statewide population by 43% between 1989 – 2012. A USFWS study of dead Bald Eagles collected throughout Maine revealed that of 127 carcasses, 14% had Pb concentrations in liver indicative of Pb poisoning (Mierzykowski et al. 2013). Sampling of free-flying resident adult Bald Eagles in north-central, western and eastern/coastal Maine by Biodiversity Research Institute (BRI) during summer months of 2015-17 revealed that none had blood Pb concentrations exceeding levels associated with clinical Pb poisoning (\geq 40 µg/dl, ww); but 40% (n = 6) had blood Pb levels exceeding those associated with subclinical Pb poisoning ($\geq 10 \ \mu g/dl$, ww). Maine wildlife rehab facilities commonly admit eagles with Pb poisoning or toxic levels of Pb in their blood.

This report summarizes findings of a study contracted by the Mi'kmaq Nation Tribe to begin establishing baseline exposure levels of Hg and Pb in resident Common Loons and Bald Eagles in the Fish River Chain of Lakes region of northeastern Maine where the headquarters the Mi'kmaq Nation Tribe (Tribe hereafter) is located.

3. **OBJECTIVES**

The objectives of this study are to:

- 1. Assess Hg and Pb exposure in resident adult Common Loons and free-flying Bald Eagles in the Fish River Chain of Lakes Study Area and begin establishing baselines of both heavy metals in these bioindicator species.
- 2. Assess potential impacts of Hg and Pb to Common Loons and Bald Eagles sampled in the Fish River Chain of Lakes Study Area.
- 3. Conduct educational outreach to hunters and anglers within the Mi'kmaq Nation Tribe and local community about the potential risks of Hg and Pb exposure in wildlife and humans.

4. STUDY AREA

We focused field efforts at lakes central to and adjacent to the Fish River Chain of Lakes located in the eastern portion of the St. John Watershed (Study Area hereafter; Figure 2). Several relatively large interconnected lakes are central to this region, including Fish River Lake, Portage Lake, Saint Froid Lake, Eagle Lake, Square Lake, Cross Lake and Long Lake. Numerous small ponds and lakes lie in the vicinity of the Fish River Chain of Lakes, including Madawaska Lake, which is hydrologically connected to the Little Madawaska River and the Aroostook River. The general Study Area is recognized for its biological richness and productive fisheries. Mixed hardwood and coniferous forests abut the majority of lakes, which span the range of residential development.

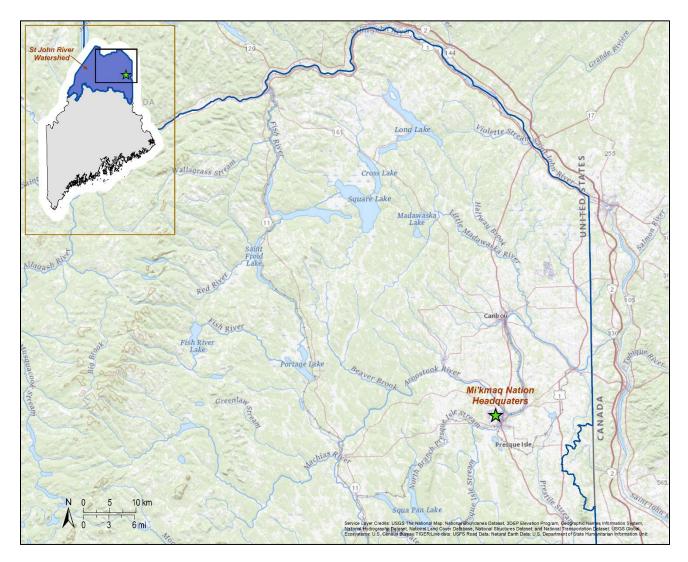


Figure 1. Fish River Chain of Lakes Study Area in northeastern Maine.

5. METHODS

5.1 Overview

In late June through mid-July, staff from Biodiversity Research Institute (BRI) and the Mi'kmaq Nation conducted boat-based surveys to locate Common Loon and Bald Eagle territories withing the Study Area, particularly those considered a part of the Fish River Chain of Lakes (e.g., Long, Mud, Cross, Square, Eagle, Portage). Findings from those surveys formed the basis for subsequent visits to Common Loon and Bald Eagle nesting territories to capture individual birds to collect biological samples for later analysis of Hg and Pb. Twenty individuals (13 Common Loons and 7 Bald Eagles) were sampled, measured, uniquely marked, and released. This report summarizes the results of those analyses. Other project components related to capacity building and education outreach are summarized elsewhere (DeSorbo 2022).

5.2 Common Loon Surveys, Capture and Sampling

We surveyed 50 sites (potential territories) for Common Loon pairs throughout the Study Area between mid June – mid July. Survey sites were selected based upon limited prior knowledge of loon pair residency and exploration of sites with a higher probability of territoriality based upon knowledge of loon habitat and territorial preferences (i.e., generally \geq 50 acres, appropriate habitat). BRI conducted a reconnaissance survey of small ponds throughout the Study Area by kayak on 15 – 18 June, and BRI and Mi'kmaq Nation staff conducted surveys of small, medium and large lakes throughout the Study Area by kayak, 12'motorboat, and 17' motorboat over the period of 28 June – 15 July, often in tandem with capture efforts. The timeframe of fieldwork was selected to target the late nesting/early hatching period of Common Loons, which coincides with the mid-late nestling period of Bald Eagles in the region. Sites occupied by Common Loon pairs were visited until the presence or presumed absence of nesting activity or young could be could be determined. Sites with loon pairs successful in hatching young were targeted for capture using a well-established nightlighting technique (Evers 2001). Once captured, each loon was fitted with a standard USGS bird band and 3 plastic color bands specifically customized for the flat tarsus of Common Loons (Photo 1). The combination of these four bands is unique to each individual, and they enable later identification in the field. Whole blood was sampled from the medial metatarsal vein of each loon and drawn into heparinized evacuated test tubes and capillary tubes in a manner consistent with previous investigations (Evers et al. 2008a). Blood samples were kept on ice until they could be frozen. Consistent with prior studies and BRI protocols, we sampled the second secondary feather from each wing of adult loons (Evers et al. 2005). Inviable eggs opportunistically encountered during surveys were placed into plastic bags, measured, and frozen.



Photo 1. Left: Blood being drawn from the leg of a banded Common Loon. Photo: Connor Stefanison. Right: A banded loon running on the surface of the water before take-off. Colored leg bands uniquely mark individual loons so they can be re-identified in the field from a distance. Photo: Daniel Poleschook

5.3 Bald Eagle Surveys, Capture and Sampling

Bald Eagles were surveyed and by boat and targeted for capture between 7 - 15 July 2021 using 2018 nest location data provided by the Maine Department of Inland Fisheries and Wildlife (MDIFW). We used a floating fish snare technique to capture individuals (Jackman et al. 1993). This technique involves capturing individuals on the surface of the water using a fish pre-fitted with monofilament nooses (Photo 2). Although we had intended to predominantly target territory-holding adults, high densities of eagles and intruders made this goal challenging. Individuals were noted as territorial in cases when observers felt confident they captured the individual associated with a particular nest (i.e., perching at nest prior to capture).





Photo 2. Left: Blood being taken from an adult Bald Eagle on Long Lake, Sinclair, Maine. Photo: Bill Hanson, BRI. Right: Floating fish snare set used to capture free-flying Bald Eagles. Photo: Chris DeSorbo, BRI.

After capture, eagles were fitted with falconer's hoods and sampled following methods outlined in DeSorbo et al. (2018). Briefly, blood was collected from the brachial vein using a 23 ¾ gauge needle and drawn into evacuated test tubes containing heparin (for sample archives or Pb analysis) and EDTA (for Pb analysis). Blood was additionally drawn into heparinized capillary tubes for Hg analysis and sample archives. Approximately four feathers were plucked from both the back and the breast of each individual and then placed into labelled envelopes. We determined sex of sampled individuals using genetics testing (Avian Biotech International, Tallahassee, Florida). All birds were banded with a standard USGS bird band on the right leg and a red alpha numeric color band (Acraft Sign and Nameplate, Edmonton, Canada) on the left leg (Photo 3).



Photo 3. Left: Banded adult Bald Eagle in flight. Unique codes on colored leg bands enable field re-identification. Credit: Kathy Gagnon Bedard. Right: Banded Bald Eagle observed in the Presque Isle area during winter. Credit: Paul Cyr

5.4 Mercury: Interpreting Tissue Concentrations and Risk

Hg in blood: Blood reflects recent dietary exposure to Hg within a timeframe of days to weeks (Evers 2018). Mercury concentrations in blood of adult birds reflect a combination of recent dietary exposure to Hg combined with that of body burdens of Hg remobilized from soft tissues (Fournier et al. 2002, Evers et al. 2005, Evers 2018). Birds have natural mechanisms of lessening the amount of their overall body burden of Hg by depurating Hg into eggs, growing feathers and other keratin-rich tissues. Birds can also detoxify MeHg through demethylation in liver and kidneys. The effects of Hg on birds are typically sublethal in nature in all but the most extreme cases. Among bird species, adverse effects of Hg are clearly defined in Common Loons. Multiple independent studies have documented Hg concentrations in blood > 3.0 μ g/g ww in association with reproductive impairment (i.e., fewer fledged young produced) (Burgess and Meyer 2008, Evers et al. 2008, Evers 2018). Numerous behavioral and physiological effects are associated with blood Hg concentrations below 3.0 μ g/g (Ackerman et al. 2016). No published study to date has found similar evidence in Bald Eagles; however, unpublished studies have noted inverse negative relationships between nestling Bald Eagle blood Hg concentrations and productivity (chicks fledged per occupied nest) in lake-dwelling Bald Eagles in Maine (DeSorbo and Evers 2007, DeSorbo et al. 2009).

Hg in feathers: Concentrations of Hg in feathers provide an index to the chronic overall body burden of Hg in adult birds and Hg exposure at the time of feather growth (Evers et al. 2005, 2008). Mercury is bound and inert in feathers after formation. The timing of feather molt or replacement is therefore important to proper interpretation of Hg exposure in birds. There is precedent in analyzing the second secondary feather in Common Loons (Evers et al. 2008). Common Loons molt and regrow their secondary feathers (which we sampled in this study) on the ocean during the winter months. Therefore, Hg concentrations in Common Loon secondary feathers reflect a combination of Hg exposure in their wintering habitat (generally low) combined with inputs of Hg remobilized from body burdens. The growth timing of individual Bald Eagle body feathers (back feathers sampled in this study) is less predictable than in loons; however, these feathers are generally considered to grow in breeding areas. Due to demonstrated differences in Hg concentrations between and among bird feathers (Peterson et al. 2019), we averaged Hg concentrations in three individual Bald Eagle back feathers for analyses in the present study. Scheuhammer (1991) suggested background Hg levels may range from $1 - 5 \mu g/g$ (in raptors), and investigations of toxic effects may be warranted in birds when feather Hg concentrations exceeded 20 μ g/g. Feather Hg concentrations >40 μ g/g were associated with increased feather asymmetry in Common Loons (Evers et al. 2008). The ability of individuals to develop symmetrical bilateral characters is considered an index to developmental stability, which can be impacted by exogenous factors such as contaminants (Clarke 1995).

Hg in Eggs: Eggs are the most sensitive life stage to environmental contaminants in birds; therefore eggs have high value in contaminant monitoring and risk assessments. Mercury concentrations in eggs reflect recent dietary exposure to Hg combined with inputs of Hg remobilized from body burdens in the laying female (Evers et al. 2003, Heinz et al. 2010). Eggs from different bird species vary in their sensitivities to Hg (Heinz et al. 2008). Eggs of birds in the *Accipitridae* family (which includes eagles) are considered to have high sensitivity, while those in the *Gavidae* family (loons) have lower Hg sensitivity (Heinz et al. 2010, Evers 2018). Lab and field studies have found that

Common Loon eggs with Hg concentrations ranging from $0.91 - 1.3 \mu g/g$ are associated with lowered hatching success and volume (Evers et al. 2003, 2008, Kenow et al. 2011).

Assessing Hg Risk: To interpret the potential risks of Hg exposure on Common Loons and Bald Eagles sampled in this study, we followed the 'Effects Concentration' (EC) approach for evaluating Hg risk described in Evers (2018). Using this approach, four Hg concentration benchmarks (EC10 – EC40) within three tissue types (blood, feather, egg) in adult piscivorous birds are associated with increasing degrees of reproductive impairment as measured in percent fewer fledged young (Table 1).

	Estimated Effect Concentrations for Hg (µg/g)				
Tissue	EC10	EC20	EC30	EC40	
Blood	1.5	2.0	2.5	3.0	
Feather	10.0	20.0	30.0	40.0	
Egg	0.48	0.65	0.80	0.98	

Table 1. Estimated Effect Concentrations in adult piscivorous birds (from Evers 2018).

^a Endpoint for impact is lowered reproductive success for all tissue types; fewer fledged young in blood and feather, and lowered hatching success in eggs. For example, tissues categorized as EC10 are associated with a 10% reduction in the number of young fledged; EC20 = 20% reduction, EC30 = 30% reduction and EC40 = 40% reduction. Hg concentrations in blood and eggs are in wet weight; concentrations in feathers are in fresh weight.

5.5 Lead: Interpreting Tissue Concentrations and Risk

Pb in blood: Blood is commonly used to evaluate Pb exposure in wild birds because it can be collected with relative ease and non-lethally compared to other tissues commonly analyzed for Pb (e.g., bone, liver, kidney). Lead concentrations in blood samples reflect Pb circulating in the bloodstream at the time of sampling. Lead concentrations in the bloodstream reflect a combination of recent exposure to Pb in addition Pb remobilized into the bloodstream from the gastrointestinal tract and other tissues such has bone (Brown et al. 2006, Fallon et al. 2017). A wide variety of factors influencing the absorption, retention and remobilization of Pb (i.e., species, anatomical characteristics of the stomach, age, gender, breeding stage, and body condition) complicate interpretation of blood Pb concentrations such that exposure levels are not consistently linked to effects and clinical signs of lead poisoning (Pain et al. 2009, Franson and Pain 2011, Fallon et al. 2017). Clinical Pb poisoning is characterized by wide variety of physical and neurological impairments; lead exposure can cause vomiting, weakness, lethargy, seizures and death (Fallon et al. 2017). Low levels of exposure to Pb are associated a wide range of negative sublethal effects including decreased immune function, neurological impairment, and adverse effects on reproduction (Burger and Gochfeld 2000).

Assessing Pb Risk: While there have been changes over time in how wildlife toxicologists interpret blood Pb concentrations (Kramer and Redig 1997, Franson and Pain 2011*a*, Fallon et al. 2017*a*, Pain et al. 2019*a*), several consistencies exist in the interpretation of several benchmark blood Pb concentrations. Birds, particularly raptors, with blood Pb concentrations >60 μ g/dl are considered

severely Pb poisoned (Franson and Pain 2011, Fallon et al. 2017, Pain et al. 2019). The blood Pb concentration of 40 μ g/dl has been commonly used as a threshold for delineating clinical Pb poisoning (Slabe et al. 2019*a*, 2022). Fallon et al. (2017b) considered raptors with blood lead concentrations between 40 μ g/dl and 60 μ g/dl to have elevated blood Pb concentrations and "potentially lethally Pb poisoned" to the extent that removal from the wild for treatment may be warranted after further evaluation.

Numerous studies considered blood Pb concentrations <20 μ g/dl to be 'background' (Brown et al. 2006, Franson and Pain 2011); however, some have proposed lowering this level to 10 μ g/dl (Church et al. 2006, Cade 2007; also see discussions and references in Fallon et al. 2017 and Pain et al. 2019). Several studies have since reported further evidence suggesting sublethal impacts of Pb occur at concentrations considerably lower than 20 μ g/dl in blood. Ecke et al. (2017) found evidence suggesting that Golden Eagles (*Aquila chrysaetos*) with blood Pb concentrations 2.5 and 4.3 μ g/dl ww exhibited 10% and 20% reduced flight heights and movement rates. Several other studies have also suggested adverse effects of Pb on physiology and body condition in waterfowl at blood Pb levels notably lower than 20 μ g/dl (Martinez-Haro et al. 2011, Newth et al. 2016). No safe blood lead concentration exists in human children. Even low concentrations of Pb in blood have been shown to adversely affect intelligence, attention span, growth, hearing, speech and other functions in children (CDC 2022). The U.S. Center for Disease Control and Prevention currently uses a blood Pb reference value (BLRV) of 3.5 μ g/dl to identify the children with higher exposure to lead than 97.5 percent of U.S. children.

For the purposes of the present study, we considered blood Pb concentrations <10 μ g/dl to represent background levels of Pb contamination and blood Pb concentrations >40 μ g/dl to be associated with clinical Pb poisoning in both Common Loons and Bald Eagles. These benchmarks mirror those used in several recent studies investigating Pb exposure in eagles and other piscivorous birds (Slabe et al. 2019*a*, 2022).

	ww)		
Tissue	Background	Subclinical Pb poisoning	Clinical Pb poisoning
Blood	<10	10 - 40	>40

Table 2. Estimated impact thresholds for Pb concentrations in bird blood.

5.6 Laboratory Analyses

Mercury (Hg): Blood, feather and egg samples collected during this study were submitted to the BRI Toxicology Lab at Biodiversity Research Institute, Portland, Maine for analysis of Hg. Tissues were analyzed for Total Hg using a Nippon MA-3000, consistent with methodology reported in previous studies (Evers et al. 2003, DeSorbo et al. 2018*b*). Similar to other studies, we considered total Hg an adequate surrogate for methylmercury (MeHg) in samples because approximately 95% of the Hg in blood, feathers and eggs is MeHg (Rimmer et al. 2005, Ackerman et al. 2016). Mercury concentrations are reported in $\mu g/g$ (ppm) as wet weight (ww) in blood and eggs and as fresh weight

(fw) for feathers. Mercury concentrations in this report should be presumed to be ww or fw within respective tissue types outlined above unless otherwise specified.

Lead (Pb): Blood samples were shipped to USGS, Forest & Rangeland Ecosystem Science Center in Boise, Idaho for sample preparation and drying prior to analysis. Dried samples were shipped to the Veterinary Diagnostic Laboratory at Michigan State University, Lansing, Michigan for Pb analysis. Details of laboratory methods for sample preparation and lead analyses generally follow those reported in (Slabe et al. 2019*a*, 2022). Blood samples with Pb concentrations below the volume-determined limit of detection of the ICP-MS are presented as below the limit of detection (LOD) as reported from the analytical laboratory.

6. **RESULTS AND DISCUSSION**

6.1 Common Loon and Bald Eagle Sampling

We captured, banded, and took biological samples from 13 adult Common Loons and 7 free-flying Bald Eagles (5 adults, 2 subadults) between 28 June and 15 July 2021 in the Study Area (Figure 2).

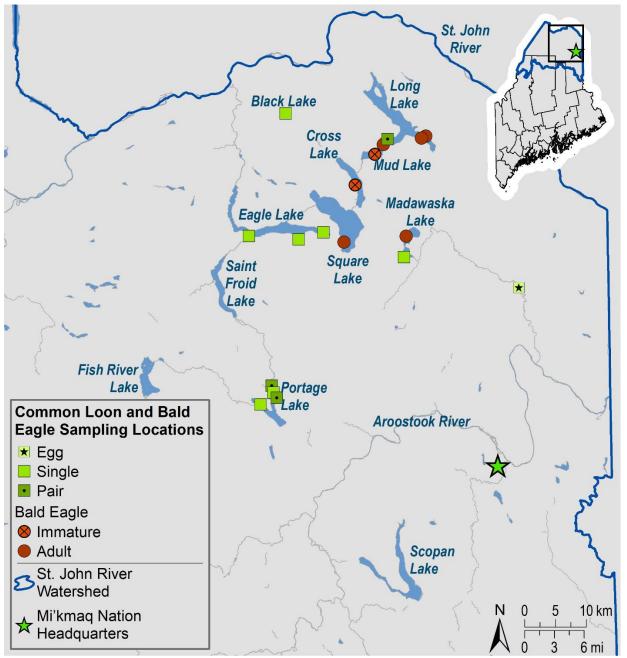


Figure 2. Sampling Locations of Common Loons and Bald Eagles within the Fish River Chain of Lakes Study Area, 2021.

Common Loons: Sampling of adult Common Loons in this study provided perspectives on Hg exposure in Common Loons within the Study Area. Direct sampling of 13 adult Common Loons and one egg during our 2021 field effort provided insights on Hg exposure at 14 nesting territories within six waterbodies (Figure 2). Lakes sampled included: Long Lake (2 adults sampled from one territory), Madawaska Lake (1 adult/territory), Eagle Lake (3 adults from three separate territories), Black Lake (1 adult/territory), Portage Lake (2 pairs and two singles representing four territories) and the Little Madawaska River (Impoundment; 1 egg) (Figure 2, Appendix 1).

Bald Eagles: We sampled at least one Bald Eagle from each of the major lakes in the northern end of the Fish River Chain of Lakes: (Long [n = 3], Mud [n = 1], Cross [n = 1], and Square [n = 1]), and an additional eagle from Little Madawaska Lake [n = 1] in the Little Madawaska River system. At least three of the adults captured (Square, Long and Madawaska) were considered to be associated with nest sites. Two adult Bald Eagles captured on Long Lake could not be confirmed as being associated with nest sites. Consistent aggregations of Bald Eagles along shorelines of most lakes in our study area such as Long Lake suggested high food availability as an attractant for Bald Eagles in this region. Subadults were captured at Mud Lake and Cross Lake. Subadult Bald Eagles have notably large home ranges; however, individuals often remain in areas of high food availability during June and July (DeSorbo et al. 2015*b*, *a*, 2020) so it is plausible that individuals sampled reflect contaminant exposure acquired within the general Study Area in recent weeks or months. All individual eagles sampled were determined to be males, except for a female sampled at Madawaska Lake. Capture and banding information for Bald Eagles captured during this study are listed in Appendix 2.

6.2 Mercury Exposure in Common Loons

Blood Hg: Blood Hg data for three previously sampled adult loons was added to our dataset for this summary (a pair of loons from Cross lake in 2003; 1 adult from Square Lake in 1997). Blood Hg concentrations of adult Common Loons sampled in our Study Area during 1997 (n = 1), 2003 (n = 2) and 2021 (n = 13) ranged from $0.56 \ \mu g/g - 1.67 \ \mu g/g$ (mean ± SD: $1.08 \pm 0.33 \ \mu g/g$, n = 16^{1}) (Figure 3). The mean Hg concentration was modestly greater in male loons ($1.15 \pm 0.33 \ \mu g/g$, n = 7) than females ($0.97 \pm 0.32 \ \mu g/g$, n = 7). The lakes sampled during these efforts provide insights on Hg exposure across a significant portion of the Fish River Chain of Lakes system (Figure 1. Figure 2).

¹ Mean across all 16 nesting territories with Hg data. Averaging Hg concentrations of individuals sampled within nesting territories and then averaged at individual lakes (to lessen potential for pseudoreplication error) had minimal changes on the mean Hg concentrations (mean \pm SD blood: 1.08 \pm 0.40, n = 7; feather: 10.9 \pm 5.32, n = 6).

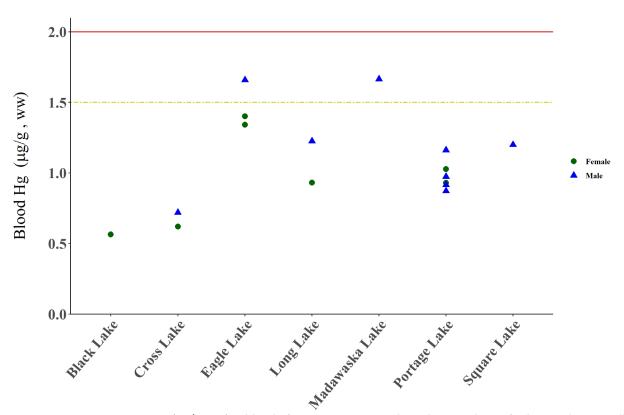


Figure 3. Mercury concentrations (μ g/g, ww) in blood of Common Loons in the Fish River Chain of Lakes Study Area. All samples were collected during the present study during 2021 except for a male sampled at Square Lake in 1997 and a pair sampled at Cross Lake in 2003 (source: BRI data).

^ Horizontal colored lines represent estimated Effect Concentration (EC) benchmarks associated with 10% (EC10, yellow line) and 20% (EC20, yellow line) reductions in reproductive success (% fewer young fledged) associated with blood Hg concentrations of 1.5 μ g/g, 2.0 μ g/g Hg in adult bird blood (Evers 2018).

Egg Hg: A single egg collected on the Little Madawaska River (Impoundment) had a Hg concentration of 0.34 μ g/g ww (1.58 μ g/g dw). Notably, this sample presumably represents a different hydrological system than the other samples collected.

Feather Hg: The feather Hg concentration for one adult Common Loon, previously sampled at Square Lake in 1997 (BRI, unpublished data), was included in this summary. Mercury concentrations in secondary feathers of Common Loons sampled in the Study Area ranged from 3.96 μ g/g (fw) to 20.23 μ g/g (mean ± SD: 9.39 ± 5.4 μ g/g, n = 14) (Figure 4). Male loons (mean ± SD: 11.81 ± 6.11, n = 8) had consistently higher feather Hg concentrations than females (6.15 ± 1.5 μ g/g, n = 6) within all lakes except for one male at Portage Lake, whose feather Hg concentration was modestly higher than one of two females sampled there (Figure 4).

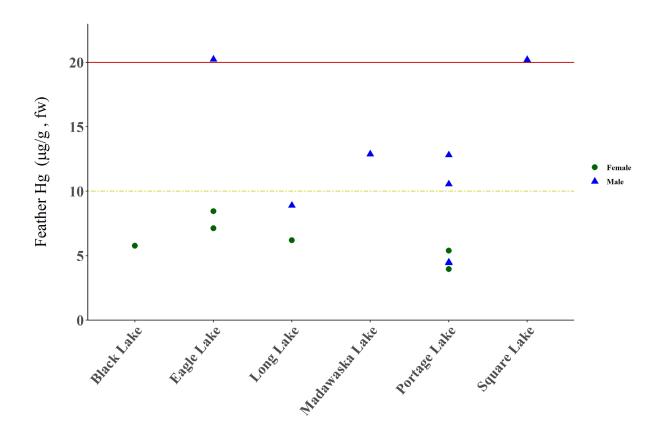


Figure 4. Mercury concentrations in secondary feathers sampled from adult Common Loons in the Fish River Chain of Lakes Study Area, 2001 (Square Lake) and 2021 (all other sites).

^ Horizontal yellow and red lines represent estimated Effect Concentration (EC) benchmarks associated with 10% (EC10, yellow line) and 20% (EC20, red line) reductions in reproductive success (% fewer young fledged) associated with adult feather Hg concentrations of 10 μg/g and 20 μg/g (Evers 2018).

Regional Comparisons

Blood Hg: The mean blood Hg concentrations of female and male Common Loons in the Study Area (females: 0.97 ± 0.32 , n = 7; males: 1.15 ± 0.33 , n = 7) appeared to be either similar or lower than the means of females and males sampled in other regions of Maine (Figure 5). Regional comparisons demonstrate that male loons consistently have greater blood Hg concentrations than female loons within subregions sampled across Maine.

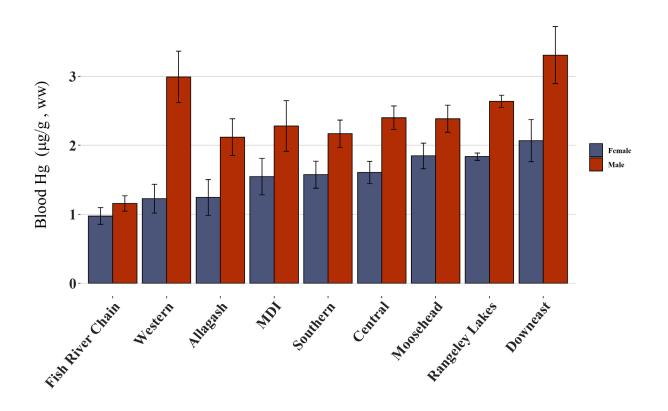


Figure 5. Mean blood mercury concentrations of Common Loons sampled in 9 different subregions of Maine, 1994-2021.

*Sample Sizes (individuals/territories/lakes): Fish River Chain (16/12/7), Western (12/6/2), Allagash (6/4/3), MDI (19/9/6), Southern (46/24/13), Central (92/60/44), Moosehead (87/46/18), Rangeley Lakes (616/150/43), Downeast (17/15/12).

Mercury exposure has been evaluated in Common Loons throughout the range of the species. Evers et al. (2008) reported that blood Hg concentrations of adult Common Loons in Maine and New Hampshire ranged from $0.13 - 11.80 \mu g/g$ (ww) and averaged $1.73 \mu g/g$ (ww). Loons residing in New England are variably exposed to Hg across sites depending on a wide variety of site-specific factors including water chemistry, water level fluctuations, differences in Hg sources and differences in the potential of lakes to produce methylmercury from Hg inputs. Blood Hg concentrations of female $(0.97 \pm 0.32, n = 7)$ and male $(1.15 \pm 0.33, n = 7)$ loons in our Study Area were lower than means reported for the Laurentian Great Lakes region (females: $1.2 \pm 1.1 \mu g/g$, ww; males: $1.8 \pm 1.4 \mu g/g$ ww) (Evers et al. 2011), and notably lower than adult loons sampled across multiple sites in Maritime Canada (females: $3.1 \mu g/g$ ww; males: $2.1 \mu g/g$, ww) (Burgess and Meyer 2008). Common Loons in the Great Lakes region have a low to moderate level of exposure to Hg, while the loon population residing in the Maritimes is among the most Hg-contaminated in the world (Burgess et al. 2005).

Egg Hg: The Hg concentration measured in the single loon egg collected in this study (0.34 μ g/g) was not notably elevated. Evers et al. (2003) analyzed inviable Common Loon eggs collected in eight U.S. states, predominantly those in the northeastern and midwestern U.S., plus Montana and Alaska.

That study found that Hg concentrations increased when moving from west to east in the U.S., and ranged from $0.07 - 4.42 \ \mu\text{g/g}$. Mean concentrations of Hg in loon eggs were lowest in Alaska (0.25 $\ \mu\text{g/g}$), while state-level means ranged from $0.41 - 0.58 \ \mu\text{g/g}$ in Minnesota, Michigan, Montana, New York and Vermont. Egg Hg concentrations were greatest in eggs from New Hampshire (0.72 $\ \mu\text{g/g}$) and Maine (0.91 $\ \mu\text{g/g}$).

Feather Hg: Feather Hg concentrations in male (mean \pm SD: 11.81 \pm 6.11 µg/g, n = 8) and female (6.15 \pm 1.5, n = 6) loons in our Study Area generally followed a similar relative geographic Hg pattern to that revealed in blood, which demonstrated generally greater mean Hg concentrations in the feathers of males than females, and also suggested loons in our Study Area may harbor similar or possibly lower Hg burdens than loons sampled in other Maine subregions. Because our sample size (of lakes and individuals) is limited within gender groups, regional Hg exposure patterns observed in this study should be considered preliminary until broader geographic sampling is conducted.

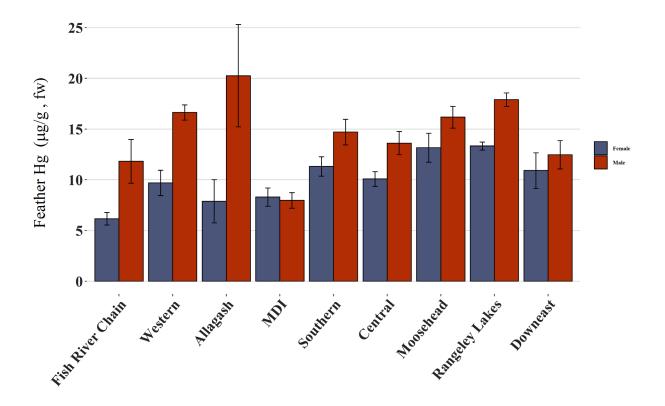


Figure 6. Mean mercury concentrations in secondary feathers of male and female Common Loons in 9 different subregions of Maine, 2001-2021.

*Sample Sizes (individuals/territories/lakes): Fish River Chain (14/11/6), Western (12/6/2), Allagash (4/3/2), MDI (14/5/4), Southern (41/19/13), Central (71/49/38), Moosehead (60/28/10), Rangeley Lakes (455/115/35), Downeast (12/10/7).

Evers et al. (1998) reported Hg concentrations in Common Loon secondary feathers collected across northern North America (Alaska, northwestern, midwestern and northeastern U.S. regions, Canadian Maritimes [NS, NB]) and found feather Hg concentrations in adult loons ranging from 2.8 to 36.7

 μ g/g. Feather Hg concentrations increased along a west to east geographic gradient, with lowest mean Hg concentrations in Alaska (males: 5.6 μ g/g; females 5.2 μ g/g) and the highest concentrations in New England (males: 15.4 μ g/g; females 10.2 μ g/g). The mean Hg concentration of feathers sampled from female loons in our Study Area was therefore similar to male and female loons sampled in Alaska, while the mean feather Hg concentration of male loons from our Study Area approached the mean of males sampled throughout New England.

Mercury Exposure Risk to Common Loons in the Study Area

Overall, a relatively small portion of Common Loons sampled in the Study Area to date were exposed to harmful concentrations of Hg (Table 3). Analyses of blood (which reflects recent Hg exposure via diet and remobilized body burdens) from resident adult loons indicated the majority of loons sampled (87.5%) were exposed to low levels of Hg, while the remainder (12.5%, n = 2) exceeded the level (EC10) associated with a 10% reduction in reproduction (i.e., fewer fledged young).

	Estimated Effect Concentration (µg/g Hg) ^a					
Tissue	<ec10< th=""><th>EC10 (1.5 μg/g)</th><th>EC20 (2.0 µg/g)</th><th>EC30 (2.5 μg/g)</th><th>EC40 (3.0 μg/g)</th></ec10<>	EC10 (1.5 μg/g)	EC20 (2.0 µg/g)	EC30 (2.5 μg/g)	EC40 (3.0 μg/g)	
Blood	87.5% (14)	12.5% (2)	0	0	0	
	<ec10< th=""><th>EC10 (10 µg/g)</th><th>EC20 (20 µg/g)</th><th>EC30 (30 µg/g)</th><th>EC40 (40 µg/g)</th></ec10<>	EC10 (10 µg/g)	EC20 (20 µg/g)	EC30 (30 µg/g)	EC40 (40 µg/g)	
Feather	65% (9)	21% (3)	14% (2)	0	0	
	<ec10< th=""><th>EC10 (0.48 µg/g)</th><th>EC20 (0.65 μg/g)</th><th>EC30 (0.80 µg/g)</th><th>EC40 (0.98 µg/g)</th></ec10<>	EC10 (0.48 µg/g)	EC20 (0.65 μg/g)	EC30 (0.80 µg/g)	EC40 (0.98 µg/g)	
Egg	100% (1)	0	0	0	0	

Table 3. Evaluation of mercury risk to adult Common Loons in the Study Area.

^a Endpoint for impact is lowered reproductive success for all tissue types; fewer fledged young in blood and feather, and lowered hatching success in eggs. Tissue concentrations for blood and egg are in wet weight, feathers in fresh weight.

Feather Hg concentrations, which reflect chronic Hg burdens in individuals, suggested that loons sampled in the Study Area were likely exposed to a sufficient amount of Hg to facilitate Hg accumulation in some individuals over time. In our sample, feather Hg concentrations fell below the EC10 threshold in 65% (n = 9) of individuals, while feather Hg concentrations fell between levels associated with EC10 and EC20 in 21% (n = 3) and 14% of individuals, respectively. No individuals harbored feather Hg concentrations exceeding 30 μ g/g fw, thus no individuals exceeded thresholds associated with EC30 or EC40.

Lastly, the Hg concentration in the single egg analyzed in the present study (0.34 μ g/g) approached, but did not exceed the Hg concentration associated with a 10% reduction in hatching success (0.48 μ g/g, fw; EC10). This egg was collected in the Little Madawaska River and is unique in our Study Area as it was collected near a former Air Force Base (Loring AFB) and it presumably reflects contamination of a different hydrological system than the other samples collected within the Fish River Chain of Lakes. The Little Madawaska River site is also unique in our sample as it is located on an impoundment. Fluctuating water levels associated with impoundments can increase Hg bioavailability to wildlife depending on a variety of factors (Snodgrass et al. 2000, DeSorbo et al. 2020*a*). Overall, we conclude that Common Loons sampled in our Study Area had relatively low overall exposure to Hg. However, male loons are clearly exposed to more Hg than females, and some individuals are exposed to a sufficient amount of Hg to facilitate accumulations over time to levels associated with adverse effects. Future expansions of sample sites to include additional waterbodies or alternative sites within large waterbodies could change the perspectives on Hg risk to Common Loons presented in this report.

6.3 Mercury Exposure in Bald Eagles

Blood Hg: Blood Hg concentrations in individual free-flying Bald Eagles captured in the Study Area ranged from $0.81 - 3.49 \ \mu$ g/g (mean ± SD: $1.73 \pm 1.02 \ \mu$ g/g, n = 7) (Figure 7). Mercury concentrations ranged more widely in adults ($0.81 - 3.49 \ \mu$ g/g, n = 5) than subadults ($1.14 - 1.25 \ \mu$ g/g, n = 2), and the mean (± SD) Hg concentration for adults ($1.95 \pm 1.15 \ \mu$ g/g, n = 5) was nearly twice the mean of subadults ($1.19 \pm 0.08 \ \mu$ g/g, n = 2). The finding of higher Hg concentrations in adult vs. subadult age classes of eagles is consistent with findings reported elsewhere (Harmata 2011, DeSorbo et al. 2018).

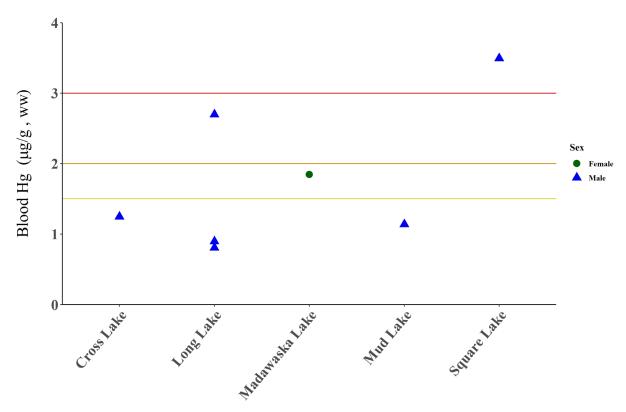


Figure 7. Mercury concentrations in blood samples collected from 7 Bald Eagles in the Fish River Chain of Lakes Study Area, 2021.

^ Horizontal colored lines represent estimated Effect Concentration (EC) benchmarks associated with 10% (EC10, yellow line) 20% (EC20, orange line) and 40% (EC40, red line) reductions in reproductive success (% fewer young fledged) associated with blood Hg concentrations of 1.5 μ g/g, 2.0 μ g/g and 3.0 μ g/g Hg in adult bird blood (Evers 2018).

Feather Hg: Mercury concentrations in Bald Eagle back feathers sampled in the present study ranged from 8.448 μ g/g (fw) to 21.553 μ g/g (fw) (mean ± SD: 17.3 ± 5.9, n = 7)² (Figure 8). The mean Hg concentration of feathers sampled from adult Bald Eagles (20.5 ± 2.6 μ g/g, fw, n = 5) was nearly twice the concentration found in subadults (9.3 ± 0.34 μ g/g, fw, n = 2).

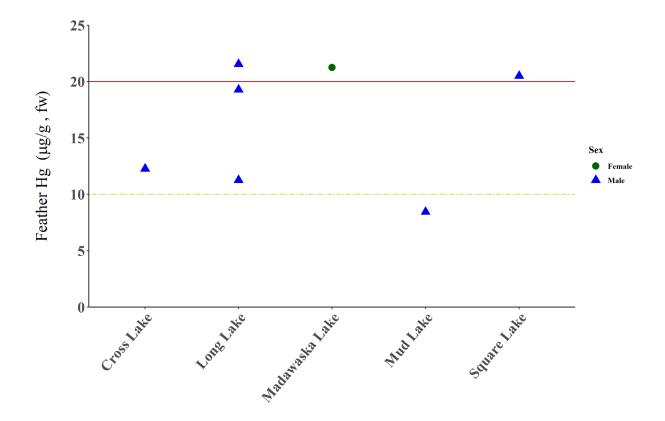


Figure 8. Mean feather mercury concentrations in 7 free-flying Bald Eagles captured in the Study Area in 2021.

^ Horizontal colored lines represent estimated Effect Concentration (EC) benchmarks associated with 10% (EC10, yellow line) and 20% (EC20, red line) reductions in reproductive success (% fewer young fledged) associated with feather Hg concentrations of 10 μ g/g and 20 μ g/g, respectively (Evers 2018). Each point is a mean of three feather Hg values.

Regional Comparisons

Small sample sizes preclude making strong conclusions about Hg exposure in the Bald Eagle population residing in the Study Area; however, this study provides insights on exposure that were previously lacking in northern Maine.

 $^{^{2}}$ Hg concentrations for 3 individual back feathers were averaged by individual for analyses (see methods). Concentrations in individual feathers ranged from 6.618 – 24.005 μ g/g (fw).

Blood Hg - Maine: Mean blood Hg concentrations of eagles sampled in three distinct regions of Maine (Fish River Chain of Lakes Study Area, Inland central/western Maine, Eastern Maine coast) differed statistically (p = 0.025, $\chi^2_2 = 7.39$, n = 33; Wilcoxon test). The mean blood Hg concentration of Bald Eagles sampled in the Fish River Chain of Lakes (mean ± SD: $1.73 \pm 1.02 \mu g/g$, ww, n = 7) was not statistically different from the mean blood Hg concentration of adults sampled along the eastern Maine coast ($2.15 \pm SD \mu g/g$, ww, n = 7); however, it was lower than the mean blood Hg concentration of adults sampled throughout inland (central and western) Maine (3.73 ± 2.1 , n = 19) (Figure 9; BRI unpublished data; DeSorbo et al. 2018). The mean blood Hg concentration of eagles in the coastal group was not statistically different from that of eagles sampled in the inland (central/western) group.

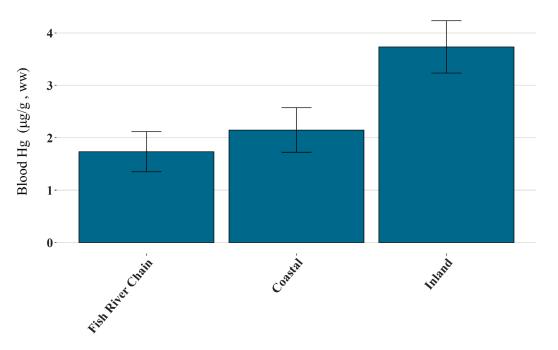


Figure 9. Mean (±SE) blood mercury concentrations in free-flying Bald Eagles sampled in three Maine regions, 2013-2021. Sample sizes: Fish River Chain (7), Coastal (7) and Inland (19).

Due in part to the challenges of efficiently capturing free-flying Bald Eagles during the breeding season, blood Hg concentrations are infrequently reported in free-flying (adult or subadult) Bald Eagles during the breeding season, and when reported, sample sizes are typically low. The mean blood Hg concentration of free-flying eagles captured in our Study Area (mean \pm SD: 1.73 \pm 1.02 μ g/g, n = 7) is lower than the means reported for several other reported populations. The mean blood Hg concentration for adults in our Study Area (1.95 \pm 1.15 μ g/g, n = 5) approaches that of resident adults sampled in the Klamath Basin and Cascades region of Oregon (2.3 μ g/g; Wiemeyer et al. 1989), a region rich in natural Hg deposits, and a series of lakes in a Hg-rich (but undisturbed) region of British Columbia used as a reference for Pinchi Lake, a highly Hg-polluted site (reference: 2.0 μ g/g). The geometric mean blood Hg concentration in lake-nesting adult Bald Eagles sampled in Maine's Penobscot River watershed, considered to be variably contaminated with Hg, was 3.16

 μ g/g³ (DeSorbo et al. 2018), while the blood Hg concentration in 3 adult Bald Eagles sampled on Pinchi Lake (BC, Canada), highly contaminated by a Hg mine, ranged from 4.7 – 9.4 μ g/g and averaged 6.7 ± 2.5 μ g/g.

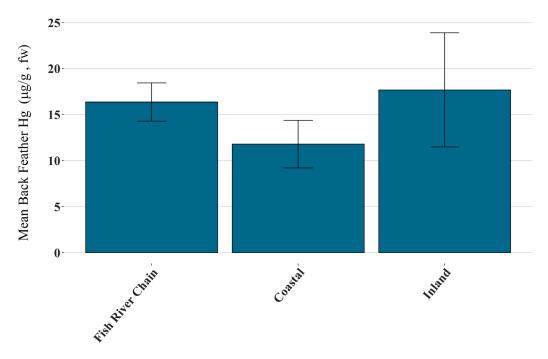


Figure 10. Mean (±SE) feather mercury concentrations from adult Bald Eagles sampled in the Fish River Chain of Lakes Study Area, coastal Maine and inland lakes in Maine (Mooselookmeguntic and Marshall Pond). Sample sizes: Fish River Chain (7), Coastal (7) and Inland (2).

Feather Hg – *Maine*: While sample sizes preclude powerful comparisons, back feather Hg concentrations did not differ between eagles sampled in the Fish River Chain of Lakes region (17.28 ±5.9 µg/g, n = 7) and those sampled along the Maine coast (11.58 ± 5.8 µg/g, n = 7) (p = 0.064, χ^2_1 = 3.4, n = 14). The Inland group (14.50 ± 4.8 µg/g, n = 2) was not statistically compared due to a limited sample size.

The mean Hg concentration of back feathers sampled from adult Bald Eagles in our Study Area (20.5 \pm 2.6 µg/g, n = 5) was intermediate among Hg concentrations reported in adult Bald Eagle feathers⁴ (see review in DeSorbo and Evers 2007), which range from 1.6 µg/g in captive eagles and 8.1 µg/g in eagles from Alaska, up to 40.8 and 43.4 µg/g at Maine Lakes, and mixed site types in New Hampshire, respectively. The mean feather Hg concentration in three adults sampled at the highly Hg-contaminated Pinchi Lake in BC was 40 \pm 22 µg/g; range: 24 - 65, n = 3 (Weech et al. 2006). The mean feather Hg concentration for adults sampled in our study was greater than the mean Hg concentration found at Lake Erie (13.0 µg/g) and similar to means for Lake Michigan, Huron,

³ Hg data for lake-nesting eagles in the Penobscot River Watershed are included in the central/western Maine Inland group used as a comparison in this study and in Figure 9.

⁴ Many studies report Hg concentrations in 2-inch distal tips of shed feathers found at nests.

Superior, and upper and lower Peninsula regions of Michigan ($20.0 - 22.0 \mu g/g$) (Bowerman et al. 1994), areas considered to have moderately elevated Hg levels in biota (Pittman et al. 2011).

Mercury Exposure Risk to Bald Eagles in the Study Area

While small sample sizes preclude making powerful conclusions, sampling in this study probably represents the first insight on Hg exposure of Bald Eagles in northern Maine. Given notably high Hg exposure has been found in adult Bald Eagles in other parts of Maine (DeSorbo et al. 2009, 2018) and portions of New York (DeSorbo et al. 2020*a*), further investigations into Bald Eagle Hg exposure in our Study Area are warranted. Evaluations of Bald Eagle tissue Hg concentrations collected in the Study Area are consistent with previous findings indicating adult eagles typically harbor higher Hg burdens than subadults or nestlings (Harmata 2011, DeSorbo et al. 2018). In our study, blood Hg concentrations were below the EC10 threshold (1.5 μ g/g) in both (100%) subadults sampled, but only 40% of adults. Blood Hg concentrations in the remaining three adults sampled were evenly distributed across EC10, EC30 and EC40 groups. The association between a blood Hg concentration of 3.0 μ g/g (EC40) and adverse reproductive impacts on reproduction endpoints (i.e., chicks fledged) has been supported by several independent studies (Burgess and Meyer 2008, Ackerman et al. 2016, Evers et al. 2008); however, no published studies have demonstrated similar impacts in Bald Eagles (but see DeSorbo et al. 2009; and discussion in DeSorbo et al. 2018). Mercury concentrations in Bald Eagle back feathers revealed patterns similar to those found in blood. Feather Hg concentrations in both subadult eagles (100%, n = 2) were below the effects concentration EC10, while concentrations in adults were distributed between EC10 (40%; n = 2) and EC20 groups (60%; n = 3). Evers et al. (2008) found that feathers of Common Loons exceeding 40 μ g/g had asymmetry in feather development, suggesting physiological impacts on development.

Tissue	<ec10< th=""><th>EC10 (1.5 µg/g)</th><th>EC20 (2.0 µg/g)</th><th>EC30 (2.5 µg/g)</th><th>EC40 (3.0 μg/g)</th></ec10<>	EC10 (1.5 µg/g)	EC20 (2.0 µg/g)	EC30 (2.5 µg/g)	EC40 (3.0 μg/g)
Blood – adult	40% (2)	20% (1)	-	20% (1)	20% (1)
Blood – subadult	100% (2)	-	-	-	-
Blood – both	57.1% (4)	14.3% (1)	-	14.3% (1)	14.3% (1)
	<ec10< th=""><th>EC10 (10 μg/g)</th><th>EC20 (20 µg/g)</th><th>EC30 (30</th><th>EC40 (40</th></ec10<>	EC10 (10 μg/g)	EC20 (20 µg/g)	EC30 (30	EC40 (40
				µg/g)	μg/g)
Feather – adults	-	40% (2)	60% (3)	-	-
Feather – subadult	100% (2)	_	_	_	-
Feather – both	28.6% (2)	28.6% (2)	42.9% (3)		

^a Endpoint for impact is lowered reproductive success for all tissue types; fewer fledged young in blood and feather, and lowered hatching success in eggs.

6.4 Mercury: Conclusions

To our knowledge, the present study is the first dedicated effort to assess Hg exposure in avian piscivores in northern Maine. The two species chosen for this study – Common Loons and Bald Eagles – are prominent and effective bioindicators for characterizing spatial and temporal contaminant patterns and related risks to wildlife. The health and viability of Common Loon and Bald Eagle populations in our Study Area are important because the region hosts northern population strongholds for both species. Both of these species are central to long-term environmental contaminant monitoring efforts.

Our sampling did not suggest that that the Common Loons we sampled were exposed to notably high levels of Hg via their recent diet. Tissue Hg concentrations fell below EC10 (concentrations exceeding EC10 represents a 10% reduction in the number of young fledged) in the majority of individuals analyzed. Feather sampling revealed that a portion of individual Common Loons sampled were exposed to a sufficient amount of Hg to enable Hg accumulation in tissues over time; however, feather Hg concentrations were below levels associated with adverse effects in a large proportion of individuals sampled.

Limited sampling of free-flying Bald Eagles revealed that some individuals in our Study Area, particularly adults, are exposed to high concentrations of Hg via their recent diet. Of the five adult and two subadult eagles sampled, two resident adults (Long Lake, nest #227C; Square Lake, nest #207G) had blood Hg concentrations exceeding 2.5 μ g/g (EC30, the level associated with a 30% reduction in young fledged) and one of those (Square Lake) had a blood Hg concentration of 3.5 $\mu g/g$, which exceeds the the EC40 level associated with a 40% reduction in young fledged (Evers 2018) and approaches the blood Hg concentration of 4.0 μ g/g associated with a wide variety of health adverse impacts (Burgess and Meyer 2008, Burgess et al. 2008). While adverse effect thresholds have not been established for adult Bald Eagles, adult Common Loons have been used as a surrogate for assessing Hg risk to other species (Ackerman et al. 2016, Evers 2018) and negative correlations between nestling blood Hg concentrations and productivity have been documented in Maine Bald Eagles (DeSorbo et al. 2009). The blood Hg concentration of 3.0 µg/g in loon blood corresponds to consumption of prey fish containing 0.23 μ g/g Hg (Depew et al. 2012), which corresponds to the USEPA human health fish consumption guideline limiting low risk consumers to 1 fish meal per week. Burgess and Meyer (2008) found that loon productivity declined by 50% when Hg concentrations in Yellow Perch were 0.21 μ g/g ww. Bald Eagle Feather Hg concentrations also showed that individuals at three sample lakes had accumulated Hg to exceed the level (20 μ g/g; Figure 8) associated with a 20% reduction in the number of young fledged, based upon Common Loon studies (Evers 2018).

It is currently unknown why Bald Eagles might be exposed to higher levels of Hg than Common Loons in our Study Area. Bald Eagles in our Study Area might be exposed to greater Hg concentrations than Common Loons due to their ability to catch larger fish (which have higher Hg levels), differences in prey selection between the two species, Hg differences among sample sites. Preliminary comparisons do not suggest that Hg differences are related to differences in sample sites since blood Hg concentrations of adult Common Loons at Long Lake and Square Lake (range: $0.93 - 1.2 \,\mu$ g/g ww, n = 3) were notably less than blood Hg concentrations of Bald Eagles sampled at those lakes (see above). The hypothesis that apparent Hg differences between the two species is a function of larger prey selection by eagles is supported by the consistent finding of higher Hg. concentrations in male loons (which are larger) than female loons in our Study Area; however, depuration of Hg into eggs by females confounds this comparison. The selection of high Hg prey by Bald Eagles is plausible given inland Maine Bald Eagles commonly eat (Todd et al. 1982) and occasionally even specialize on Chain Pickerel (CD, personal observation), which harbor high concentrations of Hg (Kamman et al. 2005, DeSorbo 2022). We suspect large chain pickerel are less likely to be targeted and successfully captured by Common Loons. Alternatively, apparent Hg differences between the two species could reflect Bald Eagles' habit of foraging away from their breeding lakes. In our field observations, however, most eagles were clearly feeding on the waterbodies upon which they were sampled, and food supply did not appear to be limited. Lastly, observed Hg differences may simply be a function of small sample sizes. A broader representation of lakes is desirable for future Hg assessments given a number of site-specific factors variably influence Hg bioavailability at lakes (i.e., water chemistry, food chain length, quantity of wetland acreage, elevation; Driscoll et al. 2007, Eagles-Smith et al. 2016, DeSorbo et al. 2020*a*).

6.5 Lead Exposure in Common Loons

Concentrations of Pb in all 13 adult Common Loon blood samples collected during this study were below the limit of detection (<100.0 - <103.6 ng/g dry weight).

Regional Comparisons and Interpretations

While blood is generally the preferred tissue type for non-lethal evaluations of Pb exposure in birds, the majority of published studies evaluating Pb exposure in Common Loons focus on sampling tissues collected post-mortem (i.e., liver, kidney, bone). As a result, limited baseline comparisons exist in the literature for Common Loon blood Pb concentrations. Burgess et al. (2005) evaluated Pb exposure in 24 adult and juvenile Common Loons in Atlantic Canada. Blood Pb concentrations were below the detection limit in 50% of individuals sampled, including all 7 of the juveniles sampled. The geometric mean Pb concentration in adult loons sampled in that study was $1.1 \,\mu$ g/dl⁵ (upper and lower SDs: $0.6 - 2.0 \mu g/dl$) and no differences were detected between males and females. Authors of that study expressed little concern about Pb exposure in that sampled population. Pokras and Chafel (1992) assessed causes of injury and mortality in 31 moribound and dead Common Loons recovered in Maine and New Hampshire. Blood Pb concentrations were evaluated in a subset of 18 individuals recovered alive, two of which had ingested lead sinkers. Of those sampled individuals, Pb concentrations ranged $1 - 5 \mu g/dl$ (mean: 2.0 $\mu g/dl$) in loons without lead sinkers (n = 16), and 78 -203 μ g/dl (mean: 140 μ g/dl) in loons that had ingested Pb sinkers (n = 2). Adult Common Loons sampled in the field in New Hampshire using a field lead analyzer (n = 51; 2012 – 2017) had a mean (\pm SD) blood Pb concentration of 1.36 \pm 2.79 μ g/dl in males and 0.72 \pm 1.28 μ g/dl in females (J. Cooley, Loon Preservation Committee, unpublished data). Brown et al. (2006) similarly used a

⁵ Concentrations in the Burgess et al. study reported in $\mu g/g$: 0.011 $\mu g/g$ (upper and lower SDs: 0.006 – 0.020 $\mu g/g$). 1 $\mu g/g$ (=ppm) = 100 $\mu g/d$ l.

portable blood lead analyzer to assess Pb exposure of Black Scoters (*Melanitta nigra*) and Steller's Eiders (*Polysticta stelleri*) in Alaska. They found that 67% of 70 seaducks sampled had blood Pb levels below the detection limit of the analyzer (< 1.4 μ g/dl), 30% had blood Pb concentrations considered a 'background' level in that study (1.5 μ g/dl – 20 μ g/dl), and 3% had blood Pb concentrations >20 μ g/dl. Authors of that study surmised that Pb exposure had occurred in the seaducks sampled, but that concentrations were below levels associated with toxicity. That study also reported higher Pb exposure in individuals sampled closer to areas of higher population density, which has been noted in other species and regions (Franson et al. 1995, Bruggeman et al. 2018).

Lead risk to Common Loons in the Study Area

All of the adult Common Loons sampled in the Study Area had blood Pb concentrations below the detection limit (Table 5). While the sample size of Common Loons sampled in the present study is relatively small and concentrated on few lakes, the lack of Pb detections in any of the 13 individuals sampled despite reasonable sample volumes (sample weight range: 0.114 - 0.389 g dry weight), suggests minimal exposure of loons we sampled to Pb⁶ through recent ingestion or remobilization from other tissues such as bone. Lead sinker (or jig) ingestion is the primary means by which Common Loons are exposed to lead (Grade et al. 2019) and thus the summer months likely represent the period associated with the highest risk of Pb exposure. Sampling in this study therefore initiates development of a useful background baseline of blood Pb concentrations in wild and presumed healthy loons that is generally lacking.

Estimated	Estimated Impact thresholds for Pb (μg/dl, ww)						
Tissue <detection background<="" th=""><th>Subclinical Pb poisoning</th><th>Clinical Pb poisoning</th></detection>			Subclinical Pb poisoning	Clinical Pb poisoning			
	limit	(<10 µg/dl)	(10 - 40 μg/dl)	(>40 µg/dl µg/dl)			
Blood	13 (100%)						

6.6 Lead Exposure in Bald Eagles

Lead was detected in four of the seven (57%) free-flying Bald Eagles sampled during this study (2 adults at Long, 1 subadult at Mud and 1 adult at Little Madawaska; range: $3.17 - 5.47 \mu g/dl$, mean⁷ ± SD: 3.95 ± 1.05 , n = 4) (Table 6). Lead was below the detection limit (<100.0 to <100.2 ng/g dry weight) for three of the seven individuals sampled (1 subadult at Cross, 1 adult at Square, and 1 of the 3 adults captured at Long Lake [the territory-holding individual]). Of the four eagles in which Pb was detected, Pb concentrations were similar between age groups (adult: mean ± SD: 4.2 ± 1.1 $\mu g/dl$, n = 3; subadult: 3.2 $\mu g/dl$, n = 1).

⁶ Despite high sample volumes; detection limits are mass-derived on a per sample basis.

⁷ Median: 3.58 μg/dl (n = 4)

Regional Comparisons

Exposure risk of Bald Eagles to Pb varies widely across populations sampled elsewhere. Caution should be exercised when making comparisons of blood Pb concentrations among studies given season (i.e., timing of sampling) and age class can strongly influence exposure (Stauber et al. 2010, Slabe et al. 2019*a*, 2022). The sampling season is important because the risk of Pb exposure is considered highest for Bald Eagles in the late fall and winter months associated with the hunting season and increased scavenging behavior by eagles (Bedrosian et al. 2012, Lindblom et al. 2017). Age class is an important factor influencing Pb exposure risk in Bald Eagles and other raptors; adults typically have higher risk than subadults, and nestlings generally have lowest risk among age groups (Bruggeman et al. 2018, Slabe et al. 2019*b*).

Slabe et al. (2019a) analyzed Pb exposure in adult Bald Eagles in Chesapeake Bay during the breeding season and found that 95% (19 of 20) had detectable levels of Pb. Mean blood Pb concentrations were higher in adults (mean: 15.37 μ g/dl, n = 20; median 7.18 μ g/dl) than non-adults (mean: 5.94 μ g/dl, n = 12). Authors of that study characterized Pb exposure to be low and considered Pb to be of little demographic consequence for that population. Miller et al. (1998) sampled fall migrant Bald Eagles aged 0.5 - 1.5 yrs of age at known Bald Eagle stopover sites in Saskatchewan, Canada (Galloway Bay, a waterfowl stopover) and Montana, U.S. (Hauser Lake, a kokanee salmon run). The median blood Pb concentration was 2.0 µg/dl for individuals sampled at Galloway Bay (n = 97), and 3.5 μ g/dl for those at Hauser Lake (n = 81). Authors of that study did not find evidence that a large portion of Bald Eagles in their study areas was exposed to notable or toxic levels of Pb, despite significant waterfowl hunting pressure at Galloway Bay. Blood Pb concentrations in subadults sampled in that study are comparable to blood Pb concentrations in subadults and adults with detectable levels of Pb in the present study. Harmata (2011) measured blood Pb concentrations in migrating Bald Eagles captured in southwest Montana near the headwaters of the Missouri River. Lead was detected in the blood of 100% of free-flying migrant eagles (42% were adults). The geometric mean blood Pb concentration was 27.2 μ g/dl (n = 88), and blood Pb concentrations declined as the season progressed from 'autumn' (1 – 22 December; 41.4 μ g/dl, n = 23) to 'winter' (23 Dec. – 29 Feb; 26.4 μ g/dl, n = 46) to 'vernal' (1 March – 15 April; 17.7 μ g/dl, n = 19). Nine percent of the birds sampled in 1 – 22 December in the Harmata study exceeded 100 μ g/dl, a level associated with acute Pb toxicity in raptors.

Maine: With the exception of those admitted to wildlife rehab facilities, a limited number of adult Bald Eagles have been evaluated for Pb exposure in New England. Mierzykowski et al. (2013) measured Pb concentrations in livers of 51 Bald Eagles and found that 16% had liver Pb concentrations indicative of Pb poisoning (>30 µg/g ww). BRI and collaborators assessed blood Pb concentrations of breeding adult Bald Eagles captured throughout central, eastern and western Maine during the summers of 2015 (n = 14) and 2016 (n = 1). Lead was detected in 87% (n = 13) of the 15 individuals sampled (range: 1.22 - 18.3 µg/dI) and the mean (± SD) blood Pb concentration in these individuals was $10.5 \pm 4.7 µg/dI$ (n = 13) (BRI, unpublished data). Of those 15 adult eagles, 47% (n = 7) had blood Pb concentrations considered 'background' (<10 µg/dI), 40% (n = 6) had blood Pb concentrations in the range associated with subclinical effects of Pb (10 – 40 µg/dI) and 13% (n = 2) had blood Pb concentrations below the detection limit. Of 11 nestling eagles aged 5 – 8.5 weeks of age sampled throughout western and north-central Maine during 2013 – 2015, Pb was detected in 45% (n = 5) of individuals. Of the nestlings with detectable Pb concentrations, blood Pb concentrations ranged from $1.4 - 9.9 \mu \text{g/dl}$ (mean ± SD: $4.4 \pm 3.7 \mu \text{g/dl}$).

Pb risk to Bald Eagles in the Study Area

The small sample size in our study precludes powerful assessments of Pb exposure risk to Bald Eagles in our Study Area; however, our sampling demonstrates that both adult and subadult Bald Eagles in our Study Area have been exposed to Pb. When detected, concentrations of Pb in our samples were notably less than benchmark levels associated with clinical Pb poisoning (>40 μ g/dl) and those associated with subclinical Pb toxicity (10 – 40 μ g/dl, ww) (Fallon et al. 2017, Slabe et al. 2019*a*, 2022).

Table 6. Evaluation of lead risk to Bald Eagles sampled in the Study Area.

Estimated Ir	Estimated Impact thresholds for Pb (μg/dl, ww)						
Tissue < detection limit		Background	Subclinical Pb poisoning Clinical Pb pois				
		(<10 µg/dl)	(10 - 40 μg/dl)	(>40 µg/dl µg/dl)			
Blood	3 (43%)	4 (57%)					

We expect that Pb exposure risk will be lowest to Maine Bald Eagles during the summer period when sampled them, and highest during the winter months due a presumed shift from a diet dominated by fish in the summer to one comprised of higher portions of carrion during the winter months (Golden et al. 2016, Buehler 2020). Numerous studies have reported higher seasonal incidences of Pb exposure in Bald Eagles during the winter months (Cruz-Martinez et al. 2012, Lindblom et al. 2017, Slabe et al. 2022). This higher incidence is typically attributed to the seasonal alignment of the sampling period with the big game hunting season (fall, early winter) in addition to seasonal increases in coyote hunting and recreational shooting of small mammals such as squirrels and racoons (Stauber et al. 2010, Bedrosian et al. 2012). The Pb concentrations revealed in our sampling therefore may represent a minimum annual baseline Pb concentration in the individuals sampled.

The sources of Pb to eagles in the present study are presently unconfirmed. While the ingestion of Pb ammunition associated with consumption of carrion containing Pb ammunition fragments is considered the primary source of Pb to Bald Eagles, other potential sources of Pb to birds exist, including ingestion of Pb paint and sediment, or inhalation of airborne particles (Katzner et al. 2017, Slabe et al. 2019*b*). Such cases are more likely to occur in industrialized regions and we presume these sources are less plausible in northeastern Maine.

6.7 Lead: Conclusions

Our study initiated efforts to assess Pb exposure in adult Common Loons and Bald Eagles in northeastern Maine during the breeding season for both species and to initiate educational outreach efforts. Lead concentrations were below the detection limit in all 13 Common Loons sampled, and 3 of the 7 Bald Eagles sampled. The timing of exposure in the Bald Eagles we sampled is unknown because complex physiological processes influence the remobilization and reabsorption of Pb (Fallon et al. 2017). Of the four eagles with detectable blood Pb levels, Pb concentrations were low, falling below the range associated with subclinical Pb poisoning ($10 - 40 \mu g/dl$) (Slabe et al. 2019*a*). Some evidence suggests sublethal effects of Pb occur below $10 \mu g/dl$ (Burger and Gochfeld 2000, Ecke et al. 2017, Fallon et al. 2017), which is consistent with guidance in the human literature, which indicates there is no safe level of Pb exposure (CDC 2022).

Sample size targets proposed in this study were necessarily small (loons: proposed 10, sampled 13. Eagles: proposed 10, sampled 7) given the measured duration of the proposed fieldwork (3 weeks; including surveys) to allow for other project needs (capacity building and outreach). Contaminant findings from this study should therefore be interpreted cautiously. Multi-year sampling in Bald Eagles is commonly required to achieve sample sizes suitable for robust analyses, particularly when targeting resident adults. Information collected in this study represents a significant step towards addressing a gap in our understanding of Pb exposure in our two focal species in this commonly overlooked region and statewide.

Investigations furthering our understanding of Pb exposure in these two iconic top predators should be recognized for their importance to conservation professionals and policymakers. Lead exposure has been implicated in lowering population resilience and population growth rates of Bald Eagle populations in the northeastern U.S. and nationwide (Hanley et al. 2022, Slabe et al. 2022). Similarly, a long-term study of New Hampshire's Common Loon population found that loon mortalities stemming from the ingestion of lead fishing gear were responsible for a 41% reduction in New Hampshire's Common Loon population between 1989 – 2012 (Grade et al. 2017). Sources of Pb to our two study species differs markedly. Whereas the predominant exposure route of lead to Bald Eagles is through the inadvertent consumption of Pb fragments remaining in animal carcass remnants, or "offal" left behind by hunters (Pain et al. 2019), Common Loons and other waterbirds are most commonly exposed to Pb by ingesting Pb fishing weights recovered from the lake bottom to aid in digestion or attached to prey fish trailing fishing gear (Grade et al. 2019).

Findings from our limited sampling did not suggest that individuals of either species we studied were exposed to high levels of Pb; however, sample sizes were small (particularly for eagles) and the seasonality of sampling must be considered during interpretation. Whereas Common Loons are primarily exposed to Pb during the summer months on their breeding grounds, the probability of Pb exposure is highest for Bald Eagles during the fall and winter months associated with game hunting seasons. No studies have evaluated exposure of Bald Eagles in our Study Area to Pb during other seasonal timeframes; however, data collected by wildlife rehabilitation facilities in Maine indicate that Pb exposure and incidences of Pb poisoning in Bald Eagles are highest during the non-summer months (Avian Haven and MDIFW, unpublished data); a finding that is consistent with numerous published studies (Stauber et al. 2010, Bedrosian et al. 2012). Despite it being the period associated with the lowest risk of Pb exposure, we assert that summer-based sampling of Bald Eagles is important because: (a) it characterizes Pb exposure in local residents and breeders, rather than individuals of unconfirmed origins typically encountered during winter months, (b) it provides perspectives on an annual baseline within individuals, and (c) there is demonstrated value in

capturing and sampling resident Bald Eagles to analyze blood samples for Hg and other contaminants (e.g., PFAS), thus simultaneous analysis of multiple contaminants is advisable.

While published reports of blood Pb concentrations are common for eagles and several waterbirds, such information remains rare for Common Loons. Our findings demonstrating that loons sampled in our Study Area were not exposed to detectable amounts of Pb are valuable in characterizing baseline blood Pb concentrations in presumed healthy adult Common Loons. Wild 'healthy' loons with elevated blood Pb levels in loons likely reflects recent Pb sinker ingestion rather than remobilization from tissues because fishing weights ingested by loons are retained in the gizzard and nearly guarantee its mortality. The probability of encountering a loon that recently swallowed a Pb sinker is likely a function of site-specific factors (i.e., fishing pressure, adoption non-Pb fishing weights by anglers) which may help inform future Pb monitoring efforts and investigations in this species.



Photo 4. Winter aggregation of Bald Eagles in Presque Isle. Credit: Paul Cyr.

7. Recommendations for Further Study

Population Monitoring – Bald Eagles: The Fish River Chain of Lakes Study Area is notable for its importance to breeding and non-breeding Bald Eagles (Photo 4). Aroosook County was notable for hosting the fastest-growing Bald Eagle population of any Maine county during the last statewide survey (MDIFW 2019). Additionally, our Study Area is well-known to attract and support large aggregations of eagles during both the summer and winter months (Photo 4). Numerous eagles

were observed along lake shorelines during our fieldwork, and large aggregations of 50 – 100 eagles – mostly subadults – are regularly observed along different sections of the the Aroostook River (Paul Cyr, personal communication). Areas that support non-breeding eagles and subadults are important to overall population stability (Penteriani et al. 2005, DeSorbo et al. 2015). Our Study Area may play an important role in supporting eagle populations in northern Maine and eastern Canada. Some well-known aggregation areas in the region such as the Presque Isle landfill (DeSorbo et al. 2020*b*) may present health hazards to eagles (Turrin et al. 2015) and probably warrant further investigation. Surveys monitoring the local resident Bald Eagle population and seasonal eagle aggregations (winter and summer) in this region would be valuable in guiding future conservation and management priorities.

Population Monitoring – Common Loons: Coarse resolution surveys conducted in the Fish River Chain of Lakes Study Area during the present study (and subsequently in 2022, see below) support speculation that our Study Area hosts a stronghold for Common Loons in northern Maine. An abundance of lakes of adquate size for loons does not necessarily imply that the region will be valuable from a demographics standoint. For example, previous surveys conducted in four Maine regions revealed that Common Loons in the nearby Allagash region exhibited lower lake occupancy rates and productivity compared to other regions surveyed (Evers et al. 2019). The Common Loon population in our Study Area is being surveyed by BRI and Tribal partners during the next three years (2023 – 2025) to support oil spill restoration efforts administered by the USFWS (Sperduto et al. 2003). Comprehensive field surveys of breeding lakes provide an important foundation for research and monitoring efforts. Color-marked individuals offer additional opportunities for long-term population monitoring and research. We recommend that Tribes, community members and stakeholders in this region consider continuing or even expanding (e.g., the Deboullie region) loon population monitoring efforts in this region after the restoration project is concluded to promote and support future research and environmental monitoring opportunities.

Continued Contaminants Monitoring in Common Loons and Bald Eagles: We recommend continuing to build sample sizes of Common Loons and Bald Eagles sampled for Pb and Hg during this study. We felt the sample sizes achieved during this study (13 loons, 1 loon egg; 7 eagles) were reasonable given the measured time (3 weeks) allocated to survey and capture fieldwork in the project proposal. Even modest additional sampling efforts boosting sample sizes of both species would substantially improve understanding of Pb and Hg exposure and risks within river and lake systems in northeastern Maine (i.e., conduct sampling of both species at Scopan, Fish River Lake, Saint Froid Lake and sample eagles along the Aroostook Madawaska Rivers). Efforts to expand sampling during the next three years (2023 – 2025) would be highly cost-effective since loon surveys prerequisite to sampling will be conducted in support of the oil spill restoration efforts noted above. Sampling of Bald Eagle nestlings (which represent contaminant exposure in the 6 – 8 weeks prior to sampling), fish, and analysis of prey remains collected at Bald Eagle nests would help elucidate why adult Bald Eagles in our study appeared to exhibit higher blood Hg levels in blood than Common Loons. Additional sampling would also enable potential assessments of additional contaminants that are of particular interest and relevance throughout Maine (e.g., PFAS). Common Loons and Bald Eagles are both optimal bioindicators for monitoring PFAS and legacy contaminants in aquatic systems (Wu et al. 2020, Dykstra et al. 2021, Grade and Vogel 2021).

Assess Wintering Bald Eagle exposure to Pb and Hg: Given large documented winter eagle aggregations in our Study Area (Photo 4) and a substantial amount of hunting activity, we recommend efforts to assess Pb and Hg exposure in Bald Eagles in our Study Area during the winter months when their risk of encountering Pb is highest and Hg is presumably lowest. Such information would complement datasets initiated in this study. Future studies could additionally consider using Pb isotopes to assess sources of Pb to individuals.

Outreach on Pb and Hg to Hunters and Anglers: Many hunters and anglers remain unaware of the threats Pb can pose to Common Loons, Bald Eagles or other organisms. Sustained efforts to conduct environmental outreach about the impacts of Pb sinkers and Pb ammunition on wildlife are needed to continue making progress on this issue. Public presentations and extensive distribution of educational materials (such as those developed through this study; see DeSorbo 2022) will be key in supporting such efforts. We recommend development and posting of educational outreach posters on Hg and Pb in key areas such as the Tribal headquarters, rod and gun clubs, sporting retailers, University of Maine Presque Isle and community gathering areas. Angling and hunting communities can be among the most valued advocates in support of wildlife and environmental health. Progress in lessening Pb poisoning in wildlife requires thoughtful and respectful engagement with these communities. Successful outreach programs implemented by other organizations can be used as a model for effective outreach in Maine (i.e., Pb sinkers, the Loon Preservation Committee, www.loon.org; Pb ammunition, The Peregrine Fund www.peregrinefund.org/lead-poisoning See lists of resources listed in DeSorbo 2022). Similar outreach efforts are also recommended to guide public decision-making about fish consumption and choices with respect to Hg contamination to avoid abandonment of this important traditional food source.

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10. **APPENDICES**

Date	Lake Name	Territory ²	Sex	Right Leg ¹	Left Leg ¹	Band Number
6/28/2021	Long Lake	Sinclair	Μ	WD/RD	OS/S	1238-04714
6/28/2021	Long Lake	Sinclair	F	B/WS	S/BS	1238-04715
6/29/2021	Black Lake	Black Lake	F	W/BS	OS/S	1238-04716
7/1/2021	Eagle Lake	East Arm	Μ	Y/GD	OS/S	1238-04717
7/1/2021	Eagle Lake	Fish River	F	R/YS	BS/S	1238-04719
7/3/2021	Madawaska Lake	McClusky Brook	Μ	B/BS	BS/S	1238-04720
7/5/2021	Portage Lake	Hutchinson Ridge	Μ	OS/G	s/os	1118-16231
7/5/2021	Portage Lake	NE Arm, Outlet	Μ	RD/O	OS/S	1238-04721
7/5/2021	Portage Lake	NE Arm, Outlet	F	BD/O	s/os	1238-04722
7/5/2021	Portage Lake	NE Arm, Outlet South	Μ	YD/G	BS/S	1238-04723
7/5/2021	Portage Lake	NE Arm "Front Country"	F	Y/GS	S/OS	1238-04724
7/5/2021	Portage Lake	NE Arm "Front Country"	Μ	RD/GD	S/BS	1238-04725
7/10/2021	Eagle Lake	Three Brooks Cove	F	YD/R	BD/S	0689-09412

Appendix 1. Capture date, lake and territory, and sex of 13 adult Common Loons captured in the Fish River Chain of Lakes Study Area.

1. Color codes are as follows: W = White; B = Blue; Y = Yellow; R = Red; O = Orange; G = Green; WD = White Dot; RD = Red Dot; GD = Green Dot; YD = Yellow Dot; WS = White Stripe; BS = Blue Stripe; YS = Yellow Stripe; OS = Orange Stripe; GS = Green Stripe; and S = Silver.

2. Territory names were given by BRI staff.

Appendix 2. Capture date, location, associated nest ID, age class, sex, and banding information of seven Bald Eagles captured in the Fish River Chain of Lakes Study Area.

Date	Lake Name	Nest ID ¹	Age Class	Sex	Red Band	Band Number
7/7/2021	Square Lake	207G	Adult	Μ	D / B	0709-03734
7/8/2021	Cross Lake		Subadult	Μ	E/A	0709-03728
7/9/2021	Long Lake	227C	Adult	Μ	K / W	0709-05956
7/9/2021	Long Lake		Adult	Μ	E / V	0709-03750
7/9/2021	Long Lake		Adult	Μ	K / V	0709-05958
7/14/2021	Mud Lake		Subadult	Μ	18/B	0709-05960
7/15/2021	L. Madawaska Lake	862A	Adult	F	K / S	0709-05957

1. Nest ID assigned by MDIFW and presumed to be associated with captured individual.