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Lead exposure biomarkers in the Common Loon

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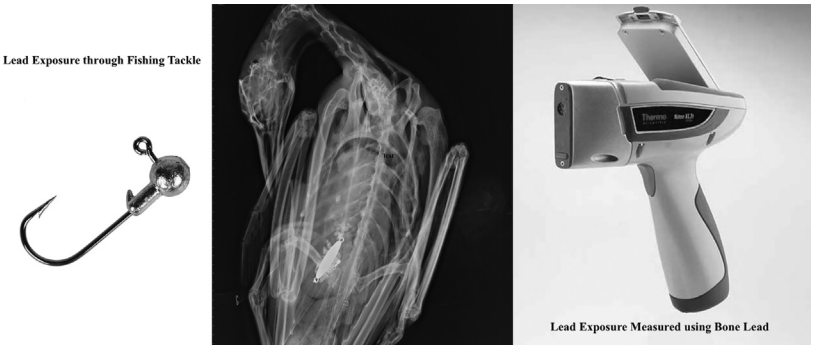
HIGHLIGHTS

- Loon bone lead can be used as a predictor of lead poisoning.
- Portable XRF can be used to assess loon bone lead in vivo in field measurements.
- Loon bone lead is a good marker of long-term aquatic health.
- There were significant differences of loon lead level by location.

GRAPHICAL ABSTRACT



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ABSTRACT

Lead in fishing tackle is a significant source of exposure to the environment, wildlife, and potentially humans. Common Loons (*Gavia immer*) are exposed to lead by eating fish which have lead tackle, or ingesting fishing weights or spent ammunition when they ingest small stones to aid in digestion. Blood lead is traditionally used as a biomarker of exposure in loons, but it only reflects recent exposures. Cumulative exposure measured via bone lead may better reflect the overall health of loons and their aquatic habitat. This study compared a portable x-ray fluorescence (XRF) device for measurement of bone lead with and without tissue overlying the bone on loon cadavers with measurements made by inductively coupled plasma mass spectrometry (ICP-MS) of different tissues from the loons. For this study we had 75 bone samples, 19 body fluid samples, and 17 liver lead samples. We found significant correlations between portable XRF bone lead measurements made with overlying tissue and ICP-MS measures of bone lead ($R = 0.88$), body fluid lead ($R = 0.65$), and liver lead ($R = 0.71$). Bone lead was found to be higher in loons collected from non-coastal regions. In assessing lead-related cause of death, bone lead proved more predictive than liver lead. Future studies should investigate the value of these biomarkers for both aquatic health and loon health to further validate our findings.

1. Introduction

Lead in fishing tackle is a significant source of exposure to the environment, wildlife, and potentially humans. Lead ingestion in Common Loons has been shown to be the leading cause of death in New Hampshire, accounting for 44% of adult loon deaths (Committee, 2013). Loons ingest the lead by eating fish that have ingested lead tackle, or

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directly by consuming the tackle, mistaking it for a pebble when they ingest small stones to aid in digestion (Committee, 2013). Additionally, loons may consume lead from spent ammunition, again, mistaking them as pebbles. Most loon mortality from lead fishing gear occurs during the peak fishing times of the summer, July through August, suggesting that it is lead deposition from current fishing activities that primarily affects loon health, rather than lost fishing gear from past years (Committee, 2013).

Loons are often considered indicators of aquatic health, as they are extremely sensitive to changes in their habitat (Evers et al., 2010; Evers et al., 2003). Contaminants that bioaccumulate, including PCBs, PBDEs, and heavy metals have been shown to be problems for loons (Evers, 2006). Since loons are representative of the overall aquatic environment, it is likely that other species that are hunted or fished are also exposed, which in turn may expose humans. Similarly, it was shown that hunters displayed increased blood lead levels in comparison to the general population, which likely arises from the use of lead bullets and shot(Hunt et al., 2009; Iqbal et al., 2009; Johansen et al., 2006). Thus, tracking lead exposure typical of aquatic species through loons will answer questions pertinent to preserving the species itself, but also for protecting the environment and human health.

Blood lead is a traditional marker of lead exposure in humans and many different animals (Beyer and Meador, 2011; Finkelstein et al., 2012; Newth et al., 2016; Rabinowitz, 1998). However, as shown in humans and other species, blood lead is only indicative of recent exposure of one week to one month, whereas the biological half-life of bone lead in humans is on the order of decades (Finkelstein et al., 2012; Rabinowitz, 1991). In human studies, it has been shown that cumulative lead exposure measured using bone lead levels is a better biomarker for studying such sublethal problems as cardiovascular disease and cognitive function (Navas-Acien et al., 2007; Shih et al., 2007; Weisskopf et al., 2004). Cumulative lifetime exposure levels would be the optimal measure to best reflect the overall health of different species and the impact of lead on the environment. Bone lead has been measured in humans using a K-shell x-ray fluorescence (KXRF) device that used a cadmium-109 radioisotope source (Chettle et al., 1991; Hu et al., 1989). Recently, a portable L-shell x-ray fluorescence (XRF) device has been used to measure bone lead in humans in vivo and condor samples (Specht et al., 2016; Specht et al., 2018; Specht et al., 2014). The portable XRF has a ten times lower measurement time than KXRF measurements and can easily be transported for site visits. This handheld device would lend itself to in-field studies of environmental contamination and cumulative lead analyses of wildlife using bone lead as a biomarker.

In pursuit of a methodology to easily identify bone lead levels, this study examined measurements of bone lead using a novel portable XRF method (Specht et al., 2016; Specht et al., 2018; Specht et al., 2014). We tested the portable XRF methods in comparison to bone measurements made using inductively coupled plasma mass spectrometry (ICP-MS). Since we had many stored carcasses of loons, we were able to test the measurement capability along with the effect of soft tissue thickness overlying the bone, which can affect the uncertainty of measurements from the device (Specht et al., 2014). We also had liver and body fluid measurements on a small number of birds, which we used for comparison between exposure assessment methods. Finally, we were able to look at differences in exposure based on location and bone, liver, and body fluid lead level associated with a clinical veterinary diagnosis of lead poisoning.

2. Methods

2.1. Loon samples

As part of a long-term, regional loon mortality study, loon cadavers were collected from Massachusetts, New Hampshire, and Maine, and were stored at Tufts Cummings School of Veterinary Medicine. Cadavers were obtained from several different sources, including the Loon Preservation Committee, WildCare, Biodiversity Research Institute, and a variety of fish and wildlife agencies. Necropsies were performed and data were obtained on age, gender, body metrics, associated pathology, and any interesting findings. Leg samples were saved both from birds that had no known lead exposure (as assayed by body fluid lead levels), and from birds with elevated blood or body fluid lead concentrations. Some leg samples were saved from birds with lead exposure seen in radiographs that showed lead fishing gear or lead shot. In those cases, the metal fragments were confirmed to be led by using LeadCheck® Swabs (3M St. Paul, MN, USA) (Buehler and Rhoda, 2012). The legs were then stored in a freezer at −40 °C until analysis. Liver samples from some of the birds were also saved and stored in the same freezer until analysis. For this study, 75 tarsometatarsus bone samples were used in the validation portion of this study. Seventeen liver samples, relating to birds that had bone measurements were then analyzed to determine the relationship between chronic and acute lead exposure. Post mortem body fluids in 19 of the birds were collected by sampling mixed fluids at the time of autopsy on the loon cadavers. Only 19 loons had sufficient fluids for sampling at that time.

2.2. Sample analysis

2.2.1. Portable X-ray fluorescence measurements

In this study, we used a portable XRF from Thermo Fisher (Niton XL3t GOLDD+, Thermo Fisher Scientific Billerica, MA). The same device was used in previous studies that made lead measurements of human and condor samples (Specht et al., 2016; Specht et al., 2018; Specht et al., 2014). We used x-ray tube settings of 50 kV 40 μA and filtration of silver and iron. The device was calibrated against known concentration doped plaster-of-Paris phantoms as done in previous works (Specht et al., 2016; Specht et al., 2014). The lead L-beta peak at 12.6 keV was used for quantification to avoid interference from peaks at the alpha region (10.55 keV). Matlab was used for spectral fitting using a Gaussian function with an exponential background fitting. The measurements were made for 3 min each at mid-length tarsometatarsus bone. Using a mid-length long bone in humans was shown to be the most consistent for comparison to cumulative lifetime exposure levels (Castellino et al., 1995; Erkkila et al., 1992). Two sets of measurements were done on each sample; one with soft tissue intact, and one after tissue had been removed. The detection limit of the device changed based on the thickness of soft tissues covering the bone being measured.

Calibration of the portable XRF was performed using lead doped plaster-of-Paris phantoms and tissue phantoms made from polymethyl methacrylate (Lucite®) as done in previous work (Specht et al., 2016; Specht et al., 2018; Specht et al., 2014). Lucite® was shown to be a good approximation to skin thickness in humans, and likely can be comparable to tissue thickness in loons as well (Specht et al., 2014).

2.2.2. Inductively coupled Plasma Mass Spectrometry Measurements

The ICP-MS measurements were made using a standard ICP mass spectrometer (NexION 350× ICP-MS, PerkinElmer Inc., Waltham, MA). A 1 cm sample of the bone from the mid-diaphysis was taken, dried in an oven, weighed out to approximately 1.0 g, and digested along with 0.04 mL of 10 ppm rhodium standard in an aluminum block heater ramping up to 130 °C in 1.5 h with continuous heat for 14–16 h in 5 mL trace metal grade nitric acid (TraceMetal grade, Fisher A509-212). Then 1 mL of 30% hydrogen peroxide (Certified A.C.S. grade. Fisher H325-500) was added to the samples. Standard quality control included initial and continuing calibration verification, reagent blanks, and a standard reference material. The ICP-MS measurements were validated with 10 subsamples from the same bones that were sent to the PADLS Toxicology Laboratory at the University of Pennsylvania School of Veterinary Medicine. Thirteen liver samples from loons for which we also had