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Total mercury in Anuran livers by Direct Solids Analysis from a Historically Industrialized Region of Northern New York (2017)

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ABSTRACT

Mercury is a highly toxic, naturally occurring element that can be readily absorbed and retained in humans and other organisms. Chemical disturbance, including heavy metal pollution, contributes to the global decline of amphibian species, which are uniquely sensitive to aquatic chemical contaminants due to their highly permeable skin and life history traits. This experiment utilized a Leco AMA254 direct mercury analyzer to quantitatively determine the mercury concentration in the livers of 22 frogs [N = 6 Green frog (*Lithobates clamitans*), N = 5 Northern Leopard frog (*Lithobates pipiens*), and N =7 American Bullfrog (*Lithobates catesbeianus*)] collected in St. Lawrence County, NY, by direct solids analysis. All the livers were found to have elevated mercury levels, some as high as 554.7 µg/kg dry mercury concentration, and none lower than 53.1µg/kg. This experiment reveals the implications of coal-burning and atmospheric mercury deposition as potential contributors to global amphibian decline. Continued industrial and agricultural activity in the region underscores the need for further research to quantify the extent of methylmercury contamination in amphibians and its ecological impacts in Northern New York and beyond.

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INTRODUCTION

One of the direct drivers contributing to amphibian decline globally is anthropogenic pollution (Egea-Serrano, 2012). Contaminants released into the environment, such as mercury (Hg), create a severe problem due to the bioaccumulation and biomagnification of methylmercury (MeHg), threatening aquatic biota (Ye, 2019). The aim of this study is to report mercury levels detected in anuran livers from a historically industrial region. Mercury is a naturally occurring metallic element that is highly toxic to wildlife, even in small quantities, exists in organic, inorganic, and elemental forms. Mercury can be detected in low environmental concentrations in air, water, soil, wildlife tissues, and through human consumable and occupational exposure pathways.

Mercury in the environment comes from both anthropogenic and natural sources. In nature, it can be found in rocks and soil, and is released into the environment by volcanic activity or weathering over time. Human activity is also responsible for significant mercury pollution, as Hg is emitted directly into the atmosphere by power plants, metal smelting, waste incineration,

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and industrial wastewater discharges in the State of New York (Wang, 2020). Atmospheric emissions have led to the deposition of Mercury in freshwater ecosystems throughout Upstate New York and are now identified as the region's dominant input of total Hg (Ye, 2019).

The form of mercury that poses the most significant hazard to living organisms and humans is organic mercury, specifically methylmercury, which is generated primarily by bacteria that perform anaerobic respiration, converting inorganic mercury. The bacteria generate methane, which can bind to mercury atoms. Mercury can become mono- or di-methylate; both forms are highly toxic. Mercury's toxicity is worsened by its ability to be readily absorbed through the skin, lungs, and digestive tract. Mercury has no recorded biological functions, making it useless to the body (Gochfeld, 2003). Environmental contaminants, including mercury exposure, can be highly detrimental to amphibians because of their permeable skin, impairing physiology, behavior, reproduction, and survival (Tornabene, 2023).

Organic mercury bioaccumulates, meaning that once the body absorbs it, the rate at which it is excreted is extremely low or zero. Bioaccumulation means that even trace amounts of mercury exposure over long periods can eventually cause the organism to accumulate damaging levels of mercury; in amphibians, the larval stage is particularly susceptible (Unrine & Jagoe, 2005). Methylmercury exists in the body as a cation that forms strong complexes with biological molecules, particularly thiols, contributing to its tendency to bioaccumulate. The abundant, highly toxic, and highly absorbable nature of methylmercury allows it to bioaccumulate at the base of the food web and biomagnify through the food chain, resulting in elevated concentrations in the tissues of fish, wildlife, and humans, causing adverse health effects (Evers, 2020). For most people who do not work with mercury or have other direct routes of exposure, such as living near a coal-burning power plant, chronic exposure can remain unknown or ignored. Once absorbed, methylmercury can easily cross the blood-brain barrier, causing cognitive impairment and damage to the central nervous system, possibly influencing amphibian behavior. Because of its widespread environmental occurrence and the tendency of organic mercury to bioaccumulate, it is found at detectable levels in most people's blood and in most wildlife (National Biomonitoring Program, 2017). Because of these biological factors, analyzing mercury in frogs is vital to understanding how heavy metals can affect biota across ecosystems.

This experiment seeks to quantify the potential of legacy mercury exposure in amphibians from a historically contaminated region, expanding a taxonomic group under-studied in the northern New York State (NYS) and Great Lakes industrial regions. The levels of organic mercury in NYS have been well documented in fish, birds, and mammals, as well as in some human studies (Evers, 2020). Such comparative wildlife studies are essential because any effects found in these species could help highlight issues related to anthropogenic pollution and introduce a legacy of further bioaccumulation across the food web. Many frog species seek habitats in or near agricultural fields and wetlands. Additionally, a wide diversity of organisms is found in such ecosystems, suggesting that the contamination of these areas can have widespread effects. Mercury is also hypothesized to have synergistic effects when combined with other toxins and toxicants (Bergeron & Bodin, 2010). The quantification of anthropogenic mercury in amphibian tissue may indicate legacy concentrations or the ability of species and environments to be restored.

MATERIALS & METHODS

Students enrolled in ENVS-370 Global Amphibian Decline at St. Lawrence University (2017; Institutional Animal Care and Use approved protocol F-17, NY State DEC wildlife permit scientific permit #1937) conducted road surveys along Park Street and Riverside Drive in Canton NY. Located in the St. Lawrence River watershed and east of the Great Lakes Region, this small rural college town is approximately 45km from the Messena superfund site; historical

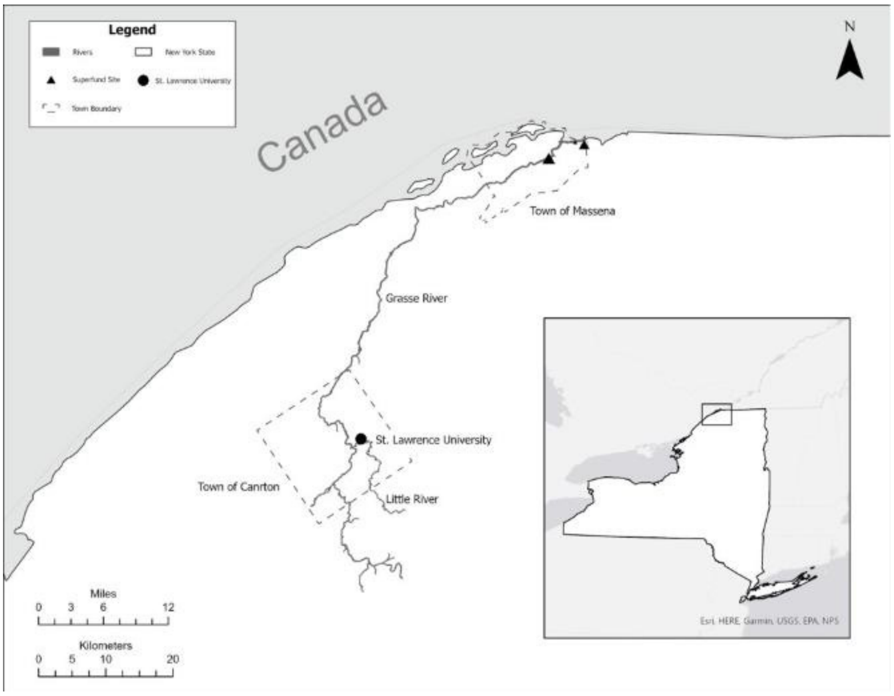

Fig. 1. Frog collection was conducted near the Little River at St. Lawrence University, which is downstream of the superfund site (Constructed using ArcGIS® software by Esri).

manufacturing and agricultural industries that discharge large amounts of pollutants into the local environment (St. Lawrence County, 2015) (Figure 1). We acknowledge the Haudenosaunee people who have traditionally occupied this land as ancestral stewards, recognizing that the land on which our surveys were done is unceded territory. We pay respect to the Six Nations Confederacy and reflect on the legacies of displacement, violence, and migration that came with colonialization (Treaty of Canandaigua, 1794)

Reflective vests, headlamps, and nitrile gloves were always worn for safety. Deceased frogs on the road and along the roadside were collected by hand and placed into Ziploc bags. Only frogs that appeared recently deceased and in good condition were collected. Each individual frog was identified by species and stage labeled accordingly, and included the date and location of collection. The labeled bags containing the frogs were placed in a freezer until were dissected.

The frogs were removed from the freezer and allowed to thaw to near room temperature before dissection. The frogs were dissected, and their whole livers were placed in labeled plastic specimen tubes and subsequently frozen again. Before analysis, the specimen tubes were transferred from the freezer to a refrigerator for 48 hours to thaw. The livers were placed in wax weigh papers and desiccated at 60°C for 15 hours and 15 minutes. At this point, the livers were dry and rigid.

The desiccation temperature of 60 °C was selected to remove moisture while minimizing analyte loss. We recognize that certain forms of mercury can volatilize at elevated temperatures. However, elemental mercury has a boiling point of 356.7 °C, and the mercury species typically present in biological tissues (primarily methylmercury and protein-bound Hg^{2+}) are strongly matrix-associated and not expected to volatilize significantly at 60 °C.

Each sample was analyzed using the Leco AMA254 Mercury Analyzer equipped with the Leco AS254 Autoloader accessory, connected to a PC running Leco Quicksilver software. The Leco AMA254 fully complies with the Environmental Protection Agency (EPA) method 7473 (1998). Briefly, samples contained in meticulously cleaned nickel sample holders are introduced into a sealed drying and combustion furnace. In this furnace, the samples are dried in a stream of oxygen before undergoing thermal decomposition. Gases generated during decomposition are carried by the oxygen stream through a catalyst furnace maintained at 750 °C, where complete decomposition occurs, and NO2, SO2, and halogens are trapped. Mercury was collected on a gold amalgamator situated at the furnace's end. Waste gases are vented from the system via the oxygen stream. The amalgamator was then heated to 500 °C to release the mercury, which was quantified using atomic absorption spectrometry. The instrument's detection limit is 0.01 ng Hg, with a working range of 0.05–600 ng Hg and repeatability of <1.5%.

To mitigate matrix interferences, the instrument was calibrated using DORM-3 (Hg = 0.382 ppm), a fish protein-certified reference material obtained from the National Research Council Canada. An 8-point calibration curve was prepared using the Leco AS254 and precisely weighed aliquots of DORM-3 (±0.1 mg).

Frog liver samples were prepared by accurately weighing approximately 0.1 g of dry liver tissue into pre-cleaned nickel sample boats using a scalpel and a stainless-steel spatula. Samples were analyzed in accordance with EPA method 7473. Two control samples of the DORM-3 standard reference material (SRM) were interspersed among the liver tissue samples and analyzed alongside them to validate the experimental method and instrument calibration. The mercury concentration determined for the DORM-3 control samples was compared to the certified value of 0.382 ppm (382 μg/kg) to ensure accuracy.

RESULTS &-DISCUSSION

The negative controls, consisting of blank sample boats, came back clean, with mercury concentrations below 0.6 μg/kg. Positive controls using DORM-3 SRM yielded an average mercury concentration of 371 μg/kg, which is within 3% of the accepted value of 382 μg/kg and well within the range of documented uncertainty, demonstrating the accuracy of the analytical method and that no drift was detected.

The frog species chosen for analysis in this study were intended to represent a wide range of organisms living in and around wetland ecosystems to determine whether these species had statistically elevated mercury levels. Groups of individuals from the three most abundant species [Green frog (*Lithobates clamitans*), Northern Leopard frog (*Lithobates pipiens*), and American Bullfrog (*Lithobates catesbeianus*)] were compared to investigate whether different frog species or the group had elevated mercury levels.

Mercury concentrations in liver tissue samples varied among the three frog species analyzed (Table 1). Green Frogs exhibited the highest mean mercury concentration (382.5 μg/kg), followed by Northern Leopard Frogs (306.1 μg/kg) and American Bullfrogs (242.4 μg/kg). However, the one-way ANOVA test found no statistically significant differences among the species (P = 0.137435, α = 0.05; Table 2).

The observed mercury levels (53.1–554.7 μg/kg) are below the FDA threshold (FDA, 2017) for mercury in marine products (1 ppm or 1000 μg/kg). However, these levels may still affect amphibian health and fitness and pose risks to predators consuming these species, potentially

Table 1. Mercury Concentrations in Liver Tissue Samples of Frog Species (Dry Weight Basis). Summary of sample sizes, total mercury concentrations, mean values, and variances for liver tissue mercury concentrations across three frog species. Liver tissue samples were collected from road-killed frogs in Canton, NY (2017).

Species	Sample Size	Avg Concentration (μg/kg)	Range (μg/kg)	Standard Deviation (μg/kg)
American Bullfrog	6	242.4	219.1 - 284.5	24.8
Green Frog	5	382.5	258.6 - 554.7	123.8
Northern Leopard Frog	7	306.1	53.1 - 433.2	380.1

Table 2. Results of the single-factor ANOVA test comparing mercury concentrations in liver tissues of three frog species, showing no statistically significant differences (P = 0.137435, α = 0.05)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	53530.94	2	26765.47	2.271956	0.137435	3.68232
Within Groups	176712.1	15	11780.81			
Total	230243	17				

amplifying mercury bioaccumulation in the food web.

Mercury concentrations in Northern New York amphibians were notably higher than those reported for similar species in other regions. For instance, California Red-Legged Frogs had significantly lower levels (48.5 µg/kg), as did Vermont Wood Frogs (44 µg/kg) (Tornabene, 2023). These elevated concentrations likely reflect regional anthropogenic pollution, particularly mercury emissions from coal-fired power plants in the Ohio River Valley (Evers, 2020). Mercury transported via atmospheric deposition settles in wetlands, where it undergoes methylation, increasing its bioavailability and uptake by organisms (Lai, Holson, & Hopke, 2007). Such a phenomenon has been studied for many years in the Adirondack State Park of New York due to high levels of acid rain caused by sulfur dioxide released from the Ohio River Valley (Acid Rain, 2017).

Historical land-use changes, such as logging in Northern New York, may have further contributed to mercury levels. Disturbance of forest ecosystems, key mercury sinks, could have released mercury into wetland environments, enhancing its availability for uptake (Brown, 2018). Forest ecosystems are essential sinks of Hg from the atmosphere; disturbances in these systems lead to mercury deposition in areas that inhibit methylmercury conversion, making mercury available for biotic uptake (Feinberg, 2024). This is consistent with elevated mercury levels observed in nearby ecosystems, such as Mudpuppy salamanders in Beauharnois, Quebec, which exhibited mercury concentrations exceeding 400 µg/kg (Gilbertson, Fox, & Bowerman, 1998)

Although atmospheric mercury deposition and a historically active logging industry could help explain the elevated mercury levels in frog species in St. Lawrence County, further study is needed. Both conclusions need to be more definitive to account for our measurements, and there is no concrete evidence of mercury-laced industrial discharges in northern New York. Massena, in the northern part of St. Lawrence County, is a known area for PCB contamination due to industrial waste from ALCOA, but not a known source of mercury [SA4] pollution, indicating that distant coal-fired power plants in the Ohio River Valley are most likely responsible for the elevated liver mercury levels measured in this experiment.

CONCLUSION

Mercury levels in the liver tissues of the analyzed frog species were elevated compared to similar species in other parts of North America. Still, they did not differ significantly among species in the samples collected from Canton, NY. These concentrations may still pose ecological risks, particularly through bioaccumulation in the food web.

Mercury pollution is a concerning environmental problem that affects ecosystem health at local, regional, and global scales, thereby adversely altering biota risk thresholds (Evers, 2020). Driven by significant anthropogenic pollution from previous and current industries in the northeast, which have contributed to mercury deposition, methylmercury is bioaccumulated across the food web in various ecosystems. Similar studies have been conducted across Upstate New York, showing elevated mercury concentrations in select bioindicators at higher trophic levels, including fish, birds, and mammals (Mercury Connections, 2019).

The findings suggest that regional anthropogenic pollution, including atmospheric mercury deposition and historical land-use changes, contributes to the elevated mercury levels observed in amphibians from Northern New York. Amphibians, a group not widely studied for mercury contamination in the United States or Northern New York, exhibit high mercury levels in their ecosystems. This is particularly concerning given their position in the food chain, as predators consuming adult frogs are likely exposed to significantly higher methylmercury levels, thereby amplifying bioaccumulation throughout the food web (Rowland, 2023). Continued industrial and agricultural activity in the region underscores the need for further research to quantify the extent of methylmercury contamination in amphibians and its ecological impacts in Northern New York and beyond.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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