Mercury Exposure in the Common Loon (*Gavia immer*) at Isle Royale National Park, Michigan

Final Report

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INTRODUCTION

Mercury in the Environment

In recent years, concentrations of mercury (Hg) in the environment have become a continent-wide issue of concern for both federal and state agencies. In Michigan, the Department of Community Health has issued a fish consumption advisory on all of the state's approximately 11,000 inland lakes. In Michigan alone, an estimated 8,400 to 10,400 pounds of Hg are released annually into the atmosphere. The primary sources of this emission include municipal waste incineration and coal combustion (Michigan Environmental Science Board 1993).

The proportion of natural versus anthropogenic Hg emissions in our environment remains largely unknown (Burgess et al. 1998a), however analysis of sediment cores indicates the current rate of Hg loading in mid-continental lakes is three to four times greater than pre-industrial levels (Swain et al. 1992). Hg atmospherically deposited on the landscape is converted by bacteria from the inorganic elemental form into methylmercury (MeHg), a more toxic organic form. MeHg is then available to the biota where it accumulates and biomagnifies in higher trophic levels animals such as predatory fish and piscivorous birds and mammals. Local watershed geology and lake chemistry play an important role in determining the amount of MeHg available to the biota. Increases in Hg are of concern because of the small margin of safety between background levels of exposure and concentrations potentially harmful to organisms including humans (Michigan Environmental Science Board 1993).

While MeHg is a potent neurotoxin at relatively low levels of exposure, determining its effects on wildlife populations is very difficult. It has been demonstrated that at high levels MeHg can have deleterious effects on the behavior and reproduction of wildlife (Barr 1986, Thompson 1996, Wiener and Spry 1996). A challenge to researchers is identifying the threshold at which Hg impairs the function of high tropic level foragers.

The Common Loon as an indicator of mercury exposure

The Common Loon (*Gavia immer*) is a piscivorous aquatic bird that nests on lakes throughout Canada and the northern tier of the United States. Formally more widespread and abundant, the Common Loon was designated a Threatened Species in Michigan in 1987 (Michigan Loon Recovery Committee 1992), with only 300-450 breeding pairs remaining. The decline of loons has been attributed to human alteration and disturbance of nesting lakes, commercial fishing nets on the Great Lakes, and environmental toxins such as mercury in the aquatic ecosystem (Caron and Robinson 1994).

The loon has been identified as a good indicator for Hg exposure based on its (1) habitat preference--lakes serving as endpoint repositories for atmospheric Hg, (2) forage base--upper trophic level organisms where Hg levels become biomagnified, (3) fidelity to breeding sites--85% of adult loons return annually to the territory they occupied the year before (Evers et al. In prep.), (4) longevity--life span of possibly over thirty years (Kaplan and Vucetich, unpubl. data), allowing documentation of Hg accumulation over time in known-aged

individuals. Additionally, Hg exposure has been correlated with reproductive impairment in the loon (Barr 1986, Burgess et al. 1998c, Meyer et al. 1998).

METHODS

Study Site

Isle Royale National Park (Keweenaw County, Michigan) is located in the northwest aspect of Lake Superior, 56 km from Thunder Bay, Canada and 96 km from Michigan's Keweenaw Peninsula. A roadless archipelago, Isle Royale is comprised of 544 km^2 of land and water containing more than 50 interior lakes. Its 740 km of Lake Superior shoreline, embodies approximately 400 outer islands. Isle Royale's many interior lakes and small islands and sheltered coves along Lake Superior provide ample breeding habitat for the Common Loon. Of the approximately 90 breeding loon pairs at Isle Royale, nearly one-third nest along the Lake Superior shoreline (Kaplan and Tischler pers. obs.), allowing for the comparative study of toxicological burdens available in Lake Superior to a variety of isolated inland lakes.

Toxicological investigations of banded loons have been conducted on Isle Royale since 1991, where a total of 24 adult and 14 juvenile loons have been sampled for Hg and other trace elements. Until 1997, monitoring efforts were limited to loons nesting and foraging in the protected coves and harbors of Lake Superior along the island's eastern end. A three-year Inland Lakes Fishery Inventory (Kallemeyn, in prep.) found Hg concentrations in northern pike at or in excess of the level of concern for human consumption (0.5 μg/g) in five of the island's interior lakes (Angleworm Lake, Lake Eva, Sargent Lake, Shesheeb Lake, and Wagejo Lake). The Michigan Fish Contaminant Monitoring Report also found elevated Hg levels in Lake Trout from Siskiwit Lake (MiDNR 1997). Following concerns raised by resource managers over these levels, monitoring efforts of loons were expanded to include inland lakes during the fall of 1997 (Sargent) and during the 1998 breeding season.

Censuses and observations

From May through September 1998, protected shoreline areas of Lake Superior (along the east end of the island) and the six interior focal lakes were surveyed for loons to determine territory occupancy, nest site locations, and productivity (hatching and fledging). One to three individuals conducted surveys using 10x42 binoculars from a canoe or kayak, or a 20-60 power spotting scope from shore. Observations were typically made at a distance of greater than 30 meters to minimize disturbance to loons. Territories on Sargent Lake and Lake Superior where juvenile loons would be captured were periodically revisited during incubation to accurately approximate the age of chicks produced.

Sample collection

From July through September, a 2-3 person team captured loons on their territories at night from a canoe (interior lakes and Lake Superior) or 14-foot aluminum boat (Lake Superior). Loons were located with a spotlight (1 million-candle power) and slowly approached. Tape-recorded or mimicked calls were used to attract loons to the canoe or boat. When in close

proximity, loons were carefully netted with a salmon-landing net, safely restrained and transported to shore where samples were collected.

Mercury exposure in loons at Isle Royale was determined through the sampling of blood and feathers from breeding adults and their offspring. The second secondary feather was chosen as the standard feather sample because it supplies a sufficient mass for analysis and has a well-defined molting pattern. Feather samples were not collected from juvenile loons captured prior to complete feather development (approx. 8 weeks of age).

Both second secondary flight feathers of adults and fully-developed juveniles were removed from each wing by cutting the calamas below the base of the feather tract. Additional contour (breast and scapular) feathers were archived for future contaminant and stable-isotope analysis. Blood samples were taken from the medial metatarsal vein using a 1- to 6-cc syringe fitted with a 20- to 25-gauge needle, and transferred into a 1- to 7-cc Vacutainer. Due to field limitations, 10% buffered formalin was added as a preservative to blood samples following U.S. Fish and Wildlife Service protocol (0.05-cc formalin: 1-cc whole blood) (Stafford and Stickel 1981, Wiemeyer et al. 1984). All loons of sufficient body size were marked with a U.S. Fish and Wildlife Service aluminum band and 1-3 colored leg bands to aid in subsequent identification in the field. Finally, each individual was bill-measured and weighed prior to being released in its respective territory. Family groups were monitored to assure adults and juveniles regrouped after capture. No injuries or fatalities occurred as a result of the capture process. All handling and sampling procedures are in accordance with guidelines provided by the Animal Behavior Society (www.animalbehavior.org/ABS/Handbook/abspolicy99.html#ethics).

Sample analysis

Secondary feathers and blood samples were analyzed for total Hg concentrations at the Animal Health Diagnostic Laboratory at Michigan State University. **Feathers** were initially washed to remove external contamination. Hg within the feathers is strongly bound to disulfide linkages of keratin (Crewther et al. 1965) and is not disturbed by washing episodes (Applequist et al. 1984) or previous environmental exposure (Goede and de Bruin 1984). Each feather was washed two times in reagent grade acetone, once in chromatography grade acetone (Burdick and Jackson, Muskegon, MI), three times in ultra pure water (4 bowl MilliQ System, Millipore Corp, Bedford, MA), one additional time in chromatography grade acetone, and then placed in a fume hood to dry overnight. Each washed feather, weighing 14-30 mg, was digested overnight at 90° C with 2 ml conc. HNO_3 (Instra-analyzed grade, J.T. Baker Inc., Phillipsburg, NJ) in a closed 30 ml Teflon container (Savillex Corp., Minnetonka, MN). The digests were quantitatively transferred to a 10 ml volumetric flask, mixed with 100 µg yttrium (JMC Specpure ICP/DCP Analytical Standards, Johnson-Matthey/Aesar, Ward Hill, MA), an internal standard, and diluted to volume. Samples were initially analyzed by inductively coupled argon plasma (ICP) emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp, Franklin, MA) (Stowe et al. 1985). An aliquot of the sample was then taken from the initial 10 ml volumetric flask and diluted an additional 100 fold for analysis of Hg by cold-vapor atomic absorption spectroscopy (LCD mercury Monitor 3200, Thermo Separation Products, Riviera Beach, FL). Accuracy was monitored by concurrent analysis of procedural blanks (in triplicate), NIST Oyster Tissue SRM 1566a with Hg certified at 0.0642 +/- 0.0067 µg/g (National Institute of Standards and

Technology, Gaithersburg, MD) and NRC Tort 2 Lobster Hepatopancreas with Hg certified at 0.27 +/- 0.06 µg/g (National Research Council of Canada, Ottawa, Canada). Each sample was finally analyzed for trace metal and mineral content again using ICP emission spectroscopy.

A 100mg (1991 – 1997 samples) to 500 mg (1998, 1999 samples) aliquot of each homogenized **whole blood** sample was digested with 2 ml conc. HNO_3 in a sealed 15 ml Teflon container overnight at 90°C. Digests were then quantitatively transferred to volumetric flasks and brought to volume with ultra pure water (100-250 ml for chicks, depending on age) and 500 ml (adults), maintaining a 2% HNO_3 solution, and analyzed as above by cold-vapor atomic absorption spectroscopy.

RESULTS

Territory occupancy and productivity

Twelve territorial loon pairs were identified on the six interior lakes with elevated fish Hg levels on Isle Royale (Table 1), however only Sargent Lake produced juvenile loons of fledging age in 1998 (n=3).

Table 1. Common Loon territory occupancy and productivity on selected interior lakes at Isle Royale NP, 1998.

Lake[a]	Territorial Pairs	Nest attempts[b]	Chicks hatched[b]	Chicks fledged
Angleworm	1	1	1	0
Eva	1	NA	0	0
Sargent	5	1,1,1,1,1	1,1,1,0,0	1,1,1,0,0
Shesheeb	1	1	NA	0
Siskiwit	4	NA	1	0
Wagejo	0	NA	0	0

[a] Lakes selected on the basis of total Hg concentrations in previously tested fish.
[b] NA indicates data on these parameters is not available.

Of the 25 loon territories monitored on Lake Superior, 12 produced chicks of fledging age (n=17) in 1998. Interior lake territories with elevated fish Hg levels hatched significantly fewer chicks than Lake Superior territories (p=0.018, Fisher Exact), however the observed difference in fledging rate was not significant (p=0.295, Fisher Exact; Table 2).

Table 2. Reproductive success of Common Loons at Isle Royale NP, 1998.

Territory Location	Lake Superior (n=25)	Interior Lakes (n=12)
Hatching/Territorial Pair	0.84	0.45
Fledging/Territorial Pair	0.48	0.25

Mercury exposure in adult loons

The mean whole blood Hg concentrations from adult loons sampled on Lake Superior (n=24 + 11 recaptures) and Sargent Lake (n=5) were 1.09 ± 0.44 (standard deviation) µg/g and 0.89 ± 0.14 µg/g, respectively. There was no significant difference in blood Hg levels between the two lakes (t=-0.993, P=0.331; Figure 1).

Mean feather Hg concentrations were similar between adults sampled on Lake Superior and Sargent Lake (Figure 2). Lake Superior adults had a mean of 10.4 ± 5.81 µg/g Hg and Sargent Lake adults averaged 10.5 ± 5.39 µg/g. Adults captured at Isle Royale ranged in feather Hg concentrations from 3.97 to 26.4 µg/g (n=43 samples). Nine of 11 (82%) adults recaptured on Lake Superior showed an increase in feather Hg. The average annual increase was 3% (1 to 4 years after initial sampling).

Male loons sampled from Lake Superior had significantly higher blood and feather Hg levels than females (blood: t=4.83, P=0.0001; feather: T=173, P=0.013; Figures 1 and 2). The mean blood Hg concentration was 0.77 ± 0.21 µg/g (n=10) in females and 1.41 ± 0.36 µg/g (n=10) in males and the mean feather Hg concentration was 8.43 ± 5.62 µg/g (n=12) in females and 12.5 ± 5.46 µg/g (n=11) in males. Lake Superior values do not include a male loon captured in Moskey Basin in 1997 and 1998 (blood Hg: 0.83/1.1 µg/g; feather Hg: 7.73/9.00 µg/g) as the breeding territory for this individual was a nearby inland lake (Wallace Lake).

Male and female adult loons on Sargent Lake had mean blood Hg levels of 0.86 µg/g (n=3) and 0.94 µg/g (n=2), respectively. Feather Hg values from Sargent Lake averaged 11.7 µg/g (n=3) in males and 8.85 µg/g (n=2) in females. There was no significant difference in Hg levels between the sexes on Sargent Lake, however sample size was limited (blood: t=-0.485, P=0.661; feather: t=0.518, P=0.640).

Mercury exposure in juvenile loons

The mean Hg concentration measured in the whole blood of **4- to 7- week-old** loon chicks sampled on Lake Superior from 1991 – 1998 was 0.06 ± 0.01µg/g (n=19). Three juvenile loons of the same age class sampled on Sargent Lake in 1998 had a mean blood Hg concentration of 0.11 ± 0.06 µg/g, nearly double the mean for Lake Superior chicks. Figure 3 shows a comparison of these levels with other sites in the Great Lakes.

Mean Hg concentrations in whole blood and secondary feathers of **fully-developed** juveniles from Isle Royale (n=15) were 0.13 ± 0.09 µg/g and 1.46 ± 0.63 µg/g, respectively. Sargent Lake juveniles (n=3) had significantly higher blood and feather Hg levels (69% higher in blood, 58% higher in feather) than Lake Superior juveniles (n=8) (blood: t=-8.25, P<0.0001; feather; T=30, P=0.0121; Figures 4 & 5). In addition, the Sargent Lake juveniles averaged 22% higher blood and 27% higher feather Hg levels than the chick from Lake Richie and 60% higher blood and 50% higher feather Hg levels than the chick from Amygdaloid Lake. There was a strong positive correlation between blood and feather Hg concentrations in fully-developed juveniles at Isle Royale (r^2=0.82, Figure 6).

Two samples from juveniles believed hatched on Wallace Lake were included in Figures 4 & 5 as inland lake samples although Lake Superior likely contributed to the Hg burdens in these individuals. In 1997, we suspect the juvenile sampled from Moskey Basin, Lake Superior was raised on Wallace Lake, and in 1998 a juvenile believed to be hatched on Wallace Lake was brought to Lake Superior at three weeks of age. The 1997 juvenile showed 56% more feather Hg than its alternate-year "sibling". Feather samples collected from juveniles in three consecutive years from Sargent Lake showed a 24% difference in Hg concentrations and siblings from Dean Island (Lake Superior) exhibited only a 16% variation in feather Hg concentrations. The instance of young loons moving overland to another waterbody is exceedingly rare (Kaplan and Tischler, pers. obs., see Appendix A for further information).

DISCUSSION

Blood as an indicator of mercury exposure

Blood provides an indicator of recent dietary Hg uptake because red blood cells (which hold nearly all Hg in blood as MeHg) have a half-life of 2 to 3 months in avian tissues (Stickel et al. 1977). We suspect that several loon pairs nesting on inland lakes at Isle Royale forage on Lake Superior. Piper et al. (1997) found most lakes within multi-lake territories were less than 0.5 kilometers apart. The ability of an adult to leave its nesting territory to forage on nearby lakes may influence Hg results in studies targeting specific waterbodies. At Isle Royale there was no significant difference in adult blood Hg levels between Sargent Lake and Lake Superior. Sargent Lake juveniles, however, showed significantly higher blood Hg concentrations than Lake Superior juveniles. Since juveniles are fed exclusively from the natal territory until fledging (Meyer et al. 1998), we identify fledging-aged juveniles as the most reliable indicator of Hg exposure in a specific waterbody.

As a whole, Isle Royale adult loons showed moderate to low blood Hg levels in comparison with other sites in the Great Lakes region (Figure 7) and North America (Figure 8).

Feathers as indicators of mercury exposure

Feathers are widely recognized as a major excretory pathway for Hg (Braune & Gaskin 1987, Burger 1993, Honda et al. 1985, Honda et al. 1986). Flight feathers sampled from adults are grown on the wintering grounds and likely reflect Hg body burdens from both summer and winter territories. Scheuhammer and Bond (1991) suggest that individuals with feather Hg concentrations >20 µg/g are at risk of toxic effects. Ten percent of adult loons sampled at Isle Royale (n=29) had feather Hg concentrations at or above this level (Figure 2). Of adult loons tested in North America 5% (n=455) exceeded the 20 µg/g risk threshold (Evers et al. 1998). Eighty-three percent adult loons recaptured at Isle Royale after their original sampling showed increases in feather Hg levels. This compares to over 70% of loons tested in North America that showed a similar pattern of accumulation (n=86) (Evers et al. 1998). It is not understood if increased feather Hg burdens are age-related or if this trend influences the population dynamics of this long-lived species.

Feather Hg levels in adult loons from Isle Royale were comparable to other sites within the Great Lakes region and North America (Figures 7 & 8).

Since the complete growth and development of juvenile flight feathers occurs on natal territories, Hg concentrations in feathers from fledging-aged juveniles reflect Hg exposure exclusively from the natal lake. At Isle Royale, the feather Hg levels found in Sargent Lake juveniles, although significantly higher than Lake Superior, are low in comparison to other North American sites (Figure 9). The strong correlation between juvenile blood and feather Hg shows that either sample matrix can be used as an indicator of Hg exposure.

Influence of weight and sex on adult loon mercury levels

Male loons across North America are significantly larger and have higher blood Hg levels than females (Evers et al. 1998). Blood Hg and weight values between the sexes differ on average 21% (Evers et al. 1998). Male loons, being larger than their female mates, may eat larger fish with greater Hg loads. Barr (1986) suggests that differences in prey species and size ranges of fish eaten by male and female loons would broaden the food base and reduce competition between pair members. Of the 52 species of fish that occurs at Isle Royale, approximately 30 species can be found within the sheltered coves and open harbors of Lake Superior, while on the inland lakes, such as Sargent, only nine species of fish are found (Lagler and Goldman 1982). If forage niche partitioning between the sexes is occurring in loons, the differences should be most pronounced in a diverse fishery where prey size and species preferences can be optimized. The 45% difference in blood Hg levels between males and females on Lake Superior is the highest found in North America. This difference may reflect the diverse fishery found in Lake Superior.

Productivity and mercury burdens

Findings from annual loon surveys conducted by NPS staff and volunteers at Isle Royale indicate an average fledging rate of 0.64 chicks per territorial pair for the entire island. Productivity at Isle Royale is slightly lower than the rate predicted by the relationship between blood Hg levels and productivity reported in Wisconsin (Meyer et al. 1998). However, at Isle Royale survey emphasis is on the number of fledging-aged young produced. Discrepancies in the number of pairs observed in early summer indicate that many territories may be failing before annual surveys are conducted, leading to a misrepresentation of actual loon productivity. Of the 41 fledging-aged juveniles recorded at Isle Royale, nine (22%) were not recorded during the standard NPS survey. Discrepancies were greatest on Lake Superior where five of 18 juveniles (27%) were not recorded during the NPS standard survey (Egan and Oelfke 1998).

Six interior lakes at Isle Royale, where fish Hg concentrations are above the advisory level set for human consumption in Michigan, had reduced productivity (hatching) in 1998. Recent studies have linked impaired reproductive effort to elevated Hg burdens in adult and juvenile loons. Barr (1986) found a strong negative correlation between the successful use of a territory by breeding loon pairs and Hg contamination (as measured in forage fish). Burgess et al. (1998c) found correlative effects between increasing Hg exposure in breeding loons and reduced hatching, but not fledging rates. We found similar results at Isle Royale when comparing loons

breeding on lakes with elevated fish Hg levels to productivity on Lake Superior. In Wisconsin, chick production was lower on lakes where blood Hg levels in 3- to 7-week-old loon chicks were elevated (Meyer et al. 1998). Mercury levels observed on Isle Royale were low compared to Wisconsin, however, Hg concentrations in juvenile loons tested on Isle Royale's interior lakes were higher than those on Lake Superior sites (Figure 3). In addition, loons from lakes with the highest fish Hg levels at Isle Royale (Eva, Shesheeb, Angleworm) were not sampled due to reproductive failure of the loons. The results of this study suggest a relationship between Hg and productivity, however, the sample size was limited so this conclusion could not be confirmed. Kallemeyn (in prep.) did not sample or compare Hg levels from 19 lakes at Isle Royale known to support breeding loons because the lakes either did not have a sport fishery (i.e. Benson, Forbes, Stickleback) or they lacked a northern pike population which serves as the basis Hg comparisons (Hatchet, Harvey, Desor). In fact, Harvey Lake had the highest fish Hg values (in yellow perch) of any lake reported in the study. Based on fish Hg results at Isle Royale additional sampling of loons on lakes not included in this study would likely indicate higher Hg levels than those reported from Sargent Lake.

Risk-assessment models are currently being developed for the Common Loon (M. Meyer and N. Burgess, pers. comm.). In the Canadian Maritimes, Hg exposure associated with a 50% impairment of loon reproduction occurred when blood Hg levels in adults were 6 µg/g and associated prey fish Hg was 0.3 µg/g. Normal loon reproduction (>0.7 chicks per pair) was found in loons that had less than 4 µg/g in the blood and less than 0.2 µg/g in the prey fish (Burgess et al. 1999). Loons tested at Isle Royale were all below impairment thresholds established in the Canadian Maritimes. However, considering the relatively low prey fish Hg levels associated with reproductive impairment, additional investigation may identify lower risk-thresholds for the species (<50% impairment), indicating a greater percentage of the loon population at some risk from Hg exposure.

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LIST OF FIGURES

APPENDIX A

In September 1997, an adult male and one juvenile loon were captured in Moskey Basin. The males' upperparts were stained red, an attribute observed only on brown-water inland lake territories (i.e. Sargent Lake and Lake Richie). In June of 1998, a pair of loons with two chicks (approximately 2 weeks old) was observed on Wallace Lake (approximately 0.4 km from Moskey Basin). One week later, a pair of loons (one identified as the 1997 male banded in Moskey Basin) was observed in Moskey Basin in the vicinity of the Wallace Lake outlet with one 2-3 week old chick and no loons remained on Wallace Lake. The banded male was recaptured in July 1998 and did not show the red stained feathers as it had one year prior. In 1999, the banded male returned and was confirmed nesting on Wallace Lake. The 1999 chick fledged Wallace Lake at 8 weeks and was attended by the adults in Moskey basin.

APPENDIX B

Subsequent to the submittal of the draft copy of this report, Hg results were updated to include samples collected in 1999 (one fully-developed juvenile from Sargent Lake and one from Caribou Island, Lake Superior). We felt that the additional information was valuable in consideration of the small sample size.