



Common Loon Eggs as Indicators of Methylmercury Availability in North America

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Abstract. Increased anthropogenic mercury (Hg) deposition since pre-industrial times, and subsequent transformation of inorganic Hg to methylmercury (MeHg) in aquatic environments, has created areas in North America where Hg poses a relatively high risk to wildlife, especially long-lived, piscivorous species. From 1995 to 2001, we opportunistically collected 577 eggs abandoned by Common Loons from eight states. Egg-Hg concentrations ranged from 0.07 to 4.42 $\mu\text{g/g}$ (ww) or 0.10 to 19.40 $\mu\text{g/g}$ (dw). Mercury was higher in eastern than in western North America. Female blood-Hg concentrations strongly correlated with those of eggs from the same territory even though the mean intraclutch Hg difference was 25%. In New England, egg volume declined significantly as egg-Hg concentrations increased. Fertility was not related to egg-Hg concentrations. Based on existing literature and this study's findings, egg-Hg risk levels were established and applied to our US data set and an existing Canadian data set. Regionally, we found the greatest risk levels in northeastern North America. With few exceptions, loon eggs are suitable indicators of methylmercury availability on lakes with territorial pairs.

Keywords: common loon; mercury; indicator; exposure; effects

Introduction

Compared to pre-industrial conditions, current levels of environmental Hg are high enough to be of potential harm to North American wildlife. The foodchain transfer of methylmercury (MeHg) through plankton (Chen et al., 2000), invertebrates (Parks and Hamilton, 1987;

Tremblay, 1999) and then fish (Wiener and Spry, 1996) provides the basis for biomagnification at a level that may be excessive, especially for obligate piscivores. Comprehensive summaries of Hg exposure in obligate piscivores have been compiled (Burger, 1993; Thompson, 1996; USEPA, 1997).

The toxicity of MeHg in birds has been extensively studied in controlled situations using captive species. However, most lab studies have used species that are not piscivorous, that likely have different sensitivities to MeHg than piscivorous species, and that therefore

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may not accurately predict the sensitivities of other species to Hg (Posthuma et al., 2002). Captive species studied include the mallard (*Anas platyrhynchos*) (Heinz, 1974, 1976, 1979), American black duck (*Anas rubripes*) (Finley and Stendell, 1978), and ring-necked pheasant (*Phasianus colchicus*) (Fimreite, 1971).

Information on the impacts of Hg on free-living bird populations has also been collected, but interpretation of the importance of Hg from these studies is often difficult because of uncertainties regarding Hg relationships with (1) tissue type (e.g., liver; Scheuhammer et al., 1998a), (2) competing inter-correlation with other contaminants (Wiemeyer et al., 1984; Henny and Herron, 1989), (3) use of unmarked individuals (Barr, 1986), (4) interspecific differences in tolerance levels, (5) possible co-accumulation of various selenium compounds (Civin-Aralar and Furness, 1991 and Bischoff et al., 2002), and (6) co-occurrence of additional ecological and/or anthropogenic stressors. Many of these difficulties can be ameliorated by examining the exposure and effects of Hg on a standard tissue for an appropriate species that occurs in habitats at high ecological risk from MeHg availability. We have chosen eggs of the common loon (*Gavia immer*) to describe and assess the risk related to MeHg exposure in freshwater piscivorous birds in North America.

The common loon inhabits lakes >6 ha across the northern tier of the United States north into Canada to the edge of the North American taiga-tundra zone. Because the loon is an upper trophic level predator, is long lived, and is an obligate piscivore that spends the breeding season on freshwater lakes, it is susceptible to the biomagnification and bioaccumulation of MeHg. Several recent field studies with the common loon provide a toxicological foundation for both Hg exposure (Burgess et al., 1998a; Evers et al., 1998; Scheuhammer et al., 1998b, 2001) and effects (Burgess et al., 1998b; Meyer et al., 1998; Nocera and Taylor, 1998; Evers et al., 2002). When the Hg exposure and effects literature is considered in combination with recently published information about loon demographics (Piper et al., 1997a; McIntyre and Evers, 2000; Evers, 2001), behavioral ecology (e.g., Evers, 1994; Gostomski and Evers, 1998), genetics (Dhar et al., 1997; Piper et al., 1997b), and population-level modeling (Evers et al., 2002), an authoritative foundation emerges for using the common loon as a primary bioindicator for the effects of contaminants such as MeHg.

Materials and methods

Egg collections

As part of various monitoring programs and studies by BioDiversity Research Institute (BRI), loon eggs were collected from eight US states (Fig. 1). Eggs were collected from nests that had been abandoned, predated or flooded. Eggs were only removed from a nest when either the adults were no longer incubating them or the eggs were determined to be nonviable (i.e., strong odor, or indications that eggs were not turned). Laying order of collected eggs was unknown.

Adult female blood collections

Female loons were live captured by night lighting and weighed (Evers, 2001). All individuals were uniquely color marked to permit future identification at a distance. Blood and feathers were collected and preserved as described in Evers et al. (1998).

Tissue processing and measurements

Egg length, width, weight, and volume were measured. Egg volume was determined by weighing water displaced by a whole egg. Egg weight was measured to the nearest 0.01 g. Fresh egg weight was determined by the weight of the water displaced by an egg (volume) and multiplying it with loon egg density (Hoyt, 1979). Loon egg density was derived from the weights of eggs characterized as infertile and sampled within the first week of incubation ($n = 183$). We considered these eggs to have minimal moisture loss. Weights of these eggs were compared to the weights of the corresponding volumes of water they displaced, which indicated that egg weights averaged 1.042 times that of their corresponding water volume weights. Eggs were scored with a scalpel, opened, and contents were placed into sterile I-Chem[®] jars. Egg contents were characterized as one of five developmental stages (Table 1). Some eggs were degraded and we were unable to confidently determine fertility (i.e., 0 rating vs. 1 rating) and these eggs were rated as "not assessable." Determining embryonic development was possible for these degraded eggs if hardened tissue or feather remnants were present.

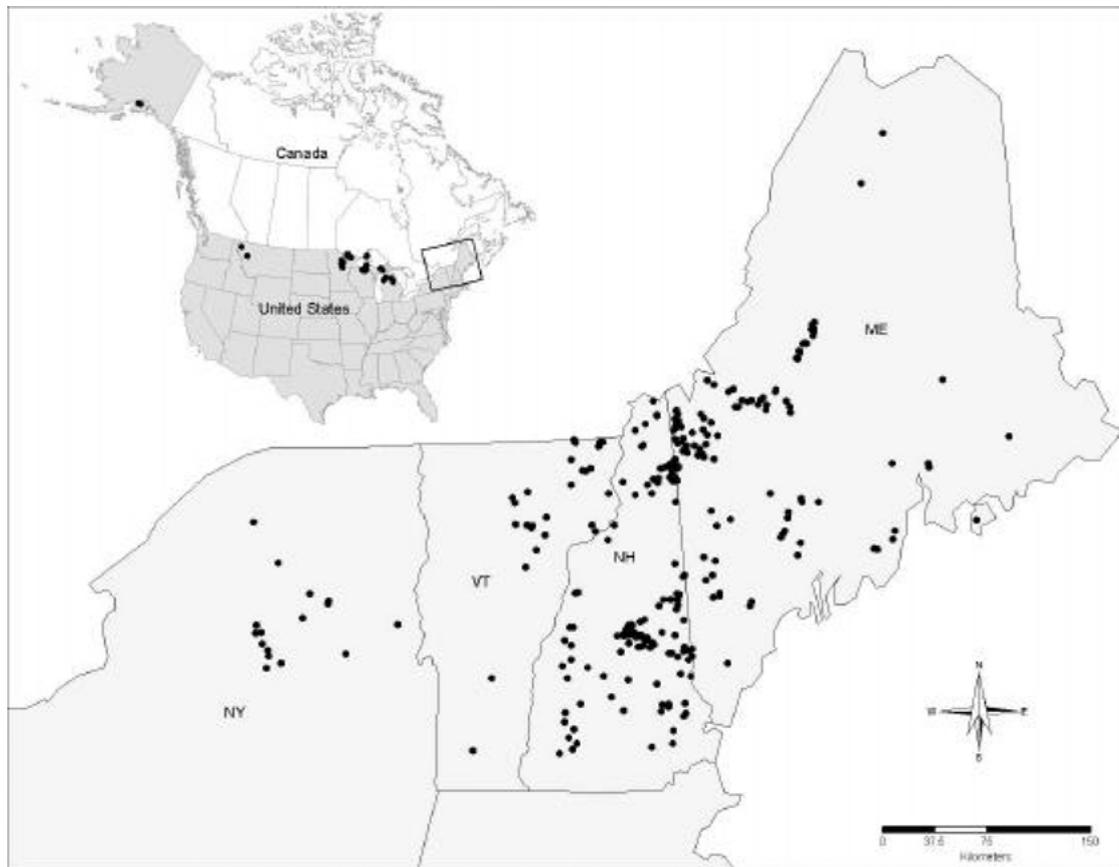


Figure 1. Locations of common loon eggs collected for mercury analysis. Inset of New England and New York provide greater detail of increased sampling efforts in that region.

Table 1. Developmental scale used for common loon embryological stage.

NA	(not assessable): Developmental stage could not be determined. Contents were gray or yellowish tan in color and typically had a foul smell. A darker color suggested some degree of development had occurred, but unless hardened tissue was detected, eggs were rated as NA.
0:	No development evident. Egg had a yellow/orange or yellow/tan yolk (intact or broken down into a liquid). A translucent jelly-like mass surrounded the yolk sac and showed no sign of embryonic development (e.g., mass not dark or hardened).
1:	Embryo was viable (length was up to 1.5 cm). The jelly-like mass (embryo) was dense and hardened. Small dark (red) eye spots were generally visible.
2:	Developing embryo had an apparent central nervous system (length was 1.5–2.0). Cranial development and recognizable eyes were apparent. Feathers were absent.
3:	The embryo showed advanced development (length was 2.0–3.0 cm). Bill was developed (e.g., egg tooth present but soft). Legs and wings were visible but not fully developed. Some feathers were present (first seen in tail).
4:	The fully developed embryo was completely covered by feathers (length >3.0 cm). Appendages were completely developed. Vent and preen gland was visible. A small portion of yolk sac remained attached to belly.

Chemical analysis

Analyses of Hg in eggs were performed at Texas A&M University Trace Element Research Laboratory (TAMU), College Station, Texas and the University of

Pennsylvania School of Veterinary Medicine (UPenn), Kennet Square, Pennsylvania. Blood and feather samples were processed and analyzed according to Evers et al. (1998). Loon eggs were received frozen in glass I-Chem jars. Samples at TAMU were

homogenized wet with an OMNI Mixer equipped with titanium blades, lyophilized in a Labconco Lyph Lock 12 freeze dryer, and powdered in a Spex Mixer Mill. Moisture was determined by weight loss upon freeze-drying. Samples at UPenn were processed wet, as received.

Samples were analyzed for Hg by cold-vapor atomic absorption (CVAA) using the method of Hatch and Ott (1968). Digestion procedures at UPenn and TAMU utilized wet and dry sample, respectively, and incorporated concentrated sulfuric and nitric acids and heating at 95 °C. Digestion at UPenn also included potassium permanganate, whereas TAMU's digestion incorporated both potassium permanganate and potassium persulfate. Following digestion, excess permanganate was reduced with hydroxylamine hydrochloride and samples were diluted to final volume (100 ml at UPenn and 50 ml at TAMU).

Following sample digestion, Hg was analyzed at UPenn using a 906GBC atomic absorption spectrometer equipped with an autosampler and hydride generator. For every 25 egg samples analyzed, a 5-point calibration curve was constructed, ranging from 10 to 100 ppb. A reagent blank consisting of 30% HCl was used to set a zero reading prior to Hg detection. A previously analyzed blood sample and certified reference material (NRCC DORM-2, 4.64 µg/gHg) were analyzed before, during, and at the end of every run to verify instrument performance. The detection limit for total Hg at UPenn was 0.25 µg/g on a wet weight basis.

Mercury content of TAMU digests was measured using an LDC Hg Monitor equipped with a 30 cm cell. One ml aliquots of sample digests were added to a closed reaction cell where the Hg²⁺ was reduced to volatile Hg⁰ with 1 ml of 10% w : v SnCl₂ in 10% HCl. Mixing and release of Hg⁰ to the headspace were facilitated by vortexing the reaction cell on a Vortex Genie 2. The headspace was introduced to the CVAA absorption cell using a syringe pump set to deliver

20 ml of deionized water to the bottom of the reaction cell. This injection approach avoided dilution of the Hg⁰ in the headspace that would have resulted from the use of a sweep gas. Calibration was accomplished using commercial standards (CPI) diluted as necessary with 5% HNO₃/1% HCl, and included a blank and five standards that ranged from 1 to 10 ppb. The calibration line was calculated using unweighted linear regression and verified using an independent standard and a blank that were analyzed following calibration, after every 10 samples, and at the end of the analysis. Hg absorbance was measured using a Shimadzu CR601 integrator in peak height mode. Each batch of Hg samples included a method blank, spiked blank, reference material (NRCC DORM-2), duplicate sample, and spiked sample (Table 2). The detection limit for total Hg at TAMU was 0.002 µg/g on a wet weight basis.

A subset of 20 loon eggs was also analyzed for MeHg at TAMU using the method of Wagemann et al. (1997). MeHg was extracted from freeze dried samples with acidic KBr and CuSO₄ solutions into a 2:3 mixture of hexane:methylene chloride. Extracted MeHg was digested and measured by element-specific detection of Hg via CVAAS, using methods similar to those described above but modified as necessary for reduced sample sizes. MeHg QC samples analyzed with loon eggs were similar to those included for total Hg analysis, with the difference that standards and spikes were prepared from CH₃HgCl (Johnson Matthey). MeHg is certified in DORM-2 at 4.47 µg/g. The detection limit for MeHg at TAMU was 0.0008 µg/g on a wet weight basis.

Dry weight vs. wet weight

Egg moisture is lost from the time the egg is laid until it hatches (Hoyt, 1979), so we analyzed 207 eggs on a dry weight (dw) basis and determined average moisture loss. Wet weights (ww) were calculated

Table 2. Quality control samples processed and analyzed with common loon eggs.

QC sample type	Total Hg average	Total Hg range	MeHg
Method blank (µg/g, ww)	0.00021	-0.00404-0.00574	0.00064
Blank spike recovery (%)	103	90-111	88
Certified reference material recovery (%)	98	89-105	87
Duplicate precision (relative % difference)	4.4	0-18	2
Spiked sample recovery (%)	101	90-110	90

using the following formula: $ww = dw \text{Hg} \times (1 - [\% \text{ moisture}/100])$ (Stickel et al., 1973). All eggs analyzed on a ww basis were adjusted for moisture loss by dividing the total egg weight by the fresh egg weight (determined by egg volume \times 1.042) and multiplying by the Hg concentration (Stickel et al., 1973). Dry weight Hg concentrations were directly determined in the lab. An approximate conversion rate to dw from ww can be based on a loon egg's average moisture content of 78.7%. Mercury levels were generally presented on a ww basis because much of the egg-Hg literature uses a ww basis. Unless otherwise noted, all egg-Hg concentrations given in the text are on a ww basis.

Statistical procedures

Mercury concentrations are expressed as arithmetic means because data were normally distributed based on normal probability plot residuals. Although geometric means are preferred for smaller data sets (Parkhurst, 1998), our large sample sizes afforded the use of arithmetic means, which are more comparable with published literature. Homoscedasticity was checked with Bartlett's test, which is sensitive to the normality assumption, and variances were generally found to be similar. Therefore log transformations were not required to stabilize variances for linear relationships. JMP software (SAS Institute, 1999) was used to test various hypotheses using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test if our ANOVA showed significant differences. Student's *t*-test was used when

comparing paired data sets. JMP software corrected for inequity of unbalanced data sets. In all cases, means were given with one standard deviation unless otherwise noted. All means are arithmetic unless otherwise stated. The level of statistical significance was defined as $p < 0.05$.

Results

Geographic variation

Mercury levels were determined for 577 eggs, collected from eight states, between 1988 and 2001 (Table 3). In the 20 eggs analyzed for MeHg, a mean of $98.6 \pm 8.2\%$ of the Hg was determined as MeHg. Mean percentage of moisture in a loon egg was not significantly different geographically among the six states with tested eggs ($F = 0.34, p > 0.05$). Mean egg-Hg concentrations were lowest in Alaska, increased eastward, and were highest in Maine. Alaska mean egg-Hg concentrations were significantly lower than those of the seven other states ($p > 0.05$ for each comparison between Alaska vs. other states using Tukey's test). Mean egg-Hg concentrations in New England ($0.77 \pm 0.54 \mu\text{g/g}$) were significantly greater than those in the Great Lakes region ($0.41 \pm 0.27 \mu\text{g/g}$) ($t = 4.35, p < 0.001$). Egg-Hg concentrations exceeding $2.0 \mu\text{g/g}$ were identified only in Maine and New Hampshire. The highest egg-Hg concentrations were from a banded female in southeastern New Hampshire that laid an egg with $3.92 \mu\text{g/g}$ in 1999 and $4.42 \mu\text{g/g}$ in 2000.

Table 3. Mercury concentrations ($\mu\text{g/g}$) wet weight (ww) and dry weight (dw) in common loon eggs collected in the United States, 1988–2001.

State	Time period	<i>n</i>	Wet weight (mean \pm SD) ¹	ww (range)	<i>n</i>	Dry weight (mean \pm SD)	dw (range)	% moisture (mean \pm SD)
Alaska	1992–1998	10	0.25 ± 0.15 (A)	0.06–0.50	0	n/a	n/a	n/a
Maine ²	1994–2001	186	0.91 ± 0.50 (B)	0.12–2.65	85	4.69 ± 2.05	0.78–4.14	78.6 ± 2.4
Michigan ³	1997–2001	24	0.54 ± 0.30 (C)	0.18–1.45	7	n/a	n/a	n/a
Minnesota ³	1997–1998	20	0.41 ± 0.26 (C)	0.10–0.89	15	1.55 ± 0.89	0.37–3.73	79.4 ± 1.4
Montana	1998–2000	5	0.43 ± 0.10 (C)	0.33–0.58	3	2.12 ± 0.63	1.55–2.80	78.3 ± 0.8
New Hampshire ²	1988–2001	263	0.72 ± 0.57 (D)	0.07–4.42	72	4.11 ± 3.14	0.82–19.40	78.7 ± 3.9
New York	1998–2001	28	0.58 ± 0.28 (C,D)	0.23–1.23	4	3.13 ± 1.51	1.58–5.08	78.6 ± 2.2
Vermont ²	1997–2001	41	0.52 ± 0.31 (C)	0.13–1.65	17	1.75 ± 0.96	0.19–4.14	76.1 ± 3.6
All states	1988–2001	577	0.55 ± 0.31	0.07–4.42	203	2.89 ± 1.30	0.19–19.4	78.7 ± 4.7

¹Mercury concentrations (ww) sharing a capital letter are not significantly different by Tukey's multiple comparison test ($p > 0.05$).

²States in New England.

³States in the Great Lakes.

Relationship between egg-Hg and female blood-Hg concentrations

We related egg-Hg concentrations with those of the blood-Hg concentrations of females captured from the same territory (Fig. 2). This relationship was initially separated into three categories according to our knowledge of the female–egg relationship. The first category included blood-Hg concentrations from color marked females captured the same year that the target egg was collected ($r^2 = 0.73$, $n = 40$). The second category was comprised of blood-Hg concentrations from color marked females that laid the target egg in a different year ($r^2 = 0.77$, $n = 24$). The third category was comprised of blood-Hg concentrations from females that were eventually captured in the same territory, but the relationship of the individual female with the target egg was not known ($r^2 = 0.74$, $n = 44$). Differences among the three categories were not significantly different ($F = 0.32$, $p > 0.05$). The relationship between egg and female blood was strongest when these three categories were combined ($r^2 = 0.79$, $n = 108$) (Fig. 2).

Intraclutch variation

Based on 86 nests where two eggs were analyzed, we found a significant difference in Hg concentrations between eggs ($F = 52.1$, $p < 0.001$). The mean difference in egg-Hg concentrations was $25 \pm 21\%$, with a range of 0–84%. Intraclutch variation in Hg

concentrations tended to increase and had a marginally significant increase between the low and extra-high Hg risk categories ($t = 1.79$, $p = 0.08$) (Table 4).

Relationship of egg size and fertility with Hg concentrations

Egg size (as measured by volume) was inversely related to egg-Hg concentrations ($t = 3.56$, $p = 0.02$) (Table 4). Eggs with very high Hg concentrations ($> 2 \mu\text{g/g ww}$) were 5% smaller than those with low Hg concentrations. Fertility and the level of embryonic development were scored for all eggs (Table 1). The Hg concentrations of infertile eggs ($0.78 \pm 0.5 \mu\text{g/g}$, $n = 201$) were not significantly different than the Hg concentrations of fertile eggs ($0.74 \pm 0.54 \mu\text{g/g}$, $n = 205$) ($t = 0.52$, $p = 0.60$) (Table 4).

Discussion

Geographic trends in egg-Hg concentrations

We found that geographic trends in egg-Hg concentrations in the US were similar to those reported for Canada (Scheuhammer et al., 2001) (Fig. 3). Our general trend of increasing egg-Hg concentrations from western to eastern North America also follows geographic trends of adult and juvenile blood-Hg concentrations previously established by Evers et al. (1998). Although atmospheric Hg deposition is

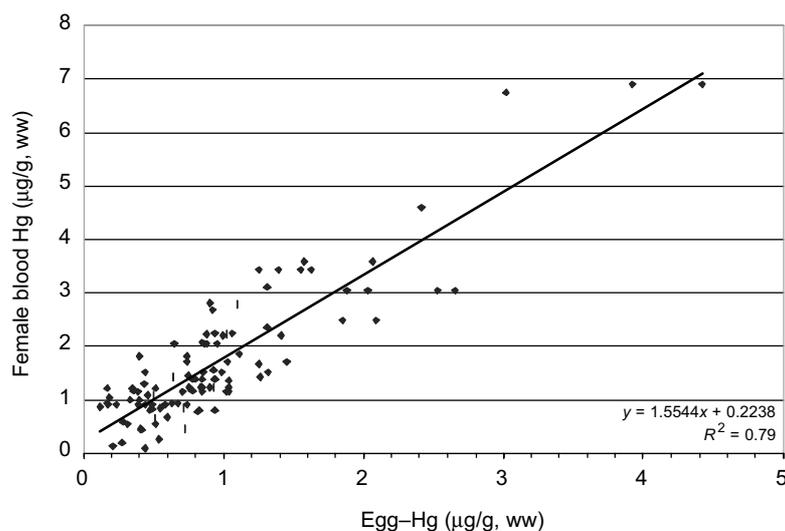


Figure 2. A comparison between Hg concentrations in common loon eggs with blood of females from the same territory.

Table 4. Estimated categories of Hg concentrations in common loon eggs and associated potential impact.

Egg-Hg levels ($\mu\text{g/g}$, ww and dw) ¹	Egg-Hg categories	Egg infertility (%) ²	Intraclutch variation (%) (mean \pm SD)	Egg volume (g) (mean \pm SD)
0–0.60 (ww) 0–2.82 (dw)	Background (low)	53 ($n = 201$)	21.5 \pm 15.8 ($n = 26$, 2-egg clutches)	149.3 \pm 14.4 ($n = 208$)
0.60–1.30 (ww) 2.82–6.10 (dw)	Elevated (moderate)	46 ($n = 157$)	24.6 \pm 20.6 ($n = 33$, 2-egg clutches)	149.0 \pm 15.0 ($n = 167$)
1.30–2.00 (ww) 6.10–9.37 (dw)	Impacting (high)	54 ($n = 35$)	31.3 \pm 24.9 ($n = 19$, 2-egg clutches)	144.4 \pm 16.0* ($n = 54$)
>2.00 (ww) >9.37 (dw)	Severe impacts (xhigh)	47 ($n = 17$)	38.0 \pm 38.2* ($n = 8$, 2-egg clutches)	141.8 \pm 14.0** ($n = 15$)

*Marginally significantly different at the $p < 0.10$ level compared to the background egg-Hg concentration.

**Significantly different at the $p < 0.05$ level compared to the background egg-Hg concentration.

¹Dry weight (dw) to wet weight (ww) conversion is based on a loon egg's average moisture content of 78.7%.

²The percent egg infertility represents eggs that were opportunistically collected from the nests that failed. This rate does not reflect egg fertility for the common loon, in general.

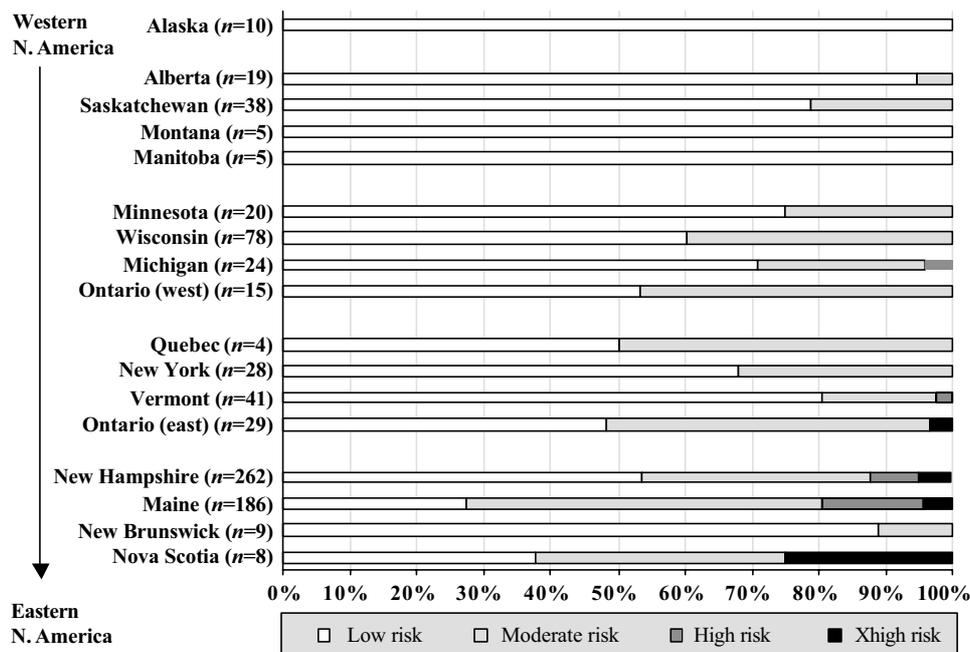


Figure 3. Proportion of common loon eggs in four Hg risk categories organized longitudinally in North America. Canadian data originated from Scheuhammer et al. (2001) and Wisconsin data from Fevold et al. (2003).

increasing in the Arctic (Lockhart et al., 1995) and seabird eggs demonstrate temporal trends of increasing Hg concentrations (Braune et al., 2001), common loon eggs from Alaska remain as our best, low Hg reference samples. Mean egg-Hg concentrations in Maine are 3.6 \times higher than levels found in Alaska.

The general geographic pattern of Hg in loon eggs that we detected is consistent with the general

atmospheric Hg deposition pattern for North America (USEPA, 1997). However, this pattern of regional atmospheric Hg input cannot explain the variability in egg and other biotic Hg concentrations that we and others have reported within regional or local areas. Some of the variability in local biotic Hg concentrations may potentially be due to differences in: (1) local anthropogenic Hg inputs from certain urban/industrial

sources (e.g., municipal incinerators, coal-burning plants, mining/smelting operations, or chlor-alkali plants); or (2) local inputs from natural geologic sources of elevated Hg (e.g., cinnabar deposits). In addition, Hg availability to biota is primarily a function of the availability of MeHg, which in turn is primarily determined by hydrological and biogeochemical factors, and thus foodchain transfer of MeHg and exposure in top predators such as loons may be elevated in some environments even in the absence of highly elevated inputs of natural or anthropogenic Hg (Watras and Huchabee, 1994; Lucotte et al., 1999). Thus, large sample sizes are required to confidently characterize the variability in biotic Hg concentrations within and between different local areas.

Relationship of Hg concentrations between female blood and eggs

A well-known depuration route for MeHg by female birds is through the egg (Braune and Gaskin, 1987; Lewis et al., 1993). In controlled studies, transfer of dietary MeHg into eggs was rapid. Kambamanoli-Dimou et al. (1991) showed that, after a single oral dose of MeHg, Hg was found in chicken eggs beginning two days after administration and peaked at 3 days, with a total of 50% of the MeHg dose being transferred to the eggs. Sell et al. (1974) showed that chicken egg-Hg concentrations declined at a rate of approximately 4% per day after dietary Hg administration had ceased. In chickens, independent of feather molts, up to 82% of Hg intake can be depurated into eggs (March et al., 1974).

A comparison of the blood-Hg concentrations of adult females with those in eggs collected from the same territory shows a highly significant positive relationship (Fig. 2). Because blood-Hg concentrations reflect dietary uptake, and eggs are a well-established depuration route for MeHg, this relationship is not unexpected. Loon egg-Hg concentrations are appropriate predictors of MeHg bioavailability at the natal lake. Potential variability in this relationship could occur due to: (1) differences in egg-Hg concentrations within a clutch, (2) differences among female loons with respect to existing Hg body burdens, and (3) use by female loons of multi-lake territories for foraging. Further explanation of these potential confounding factors follows.

Although extrapolation of egg contaminant burdens from the analysis of one egg to the remainder of the clutch is still considered standard for organochlorines (Custer et al., 1990), recent evidence for significant intraclutch variation in egg-Hg concentrations indicates that a knowledge of laying order may be required to make more accurate predictions (Morera et al., 1997). Intraclutch variability occurs for Hg concentrations in bird eggs because the first egg laid typically has higher concentrations than those following (Becker and Sperveslage, 1989; Becker, 1992). In 3-egg clutches of herring gulls (*Larus argentatus*) and common terns (*Sterna hirundo*), Becker (1992) found Hg concentrations in the first eggs laid were, respectively, 39 and 37% higher than in the last egg. In our study, mean intraclutch differences for loon egg-Hg concentrations were $25 \pm 21\%$ (CI = 4–46%). However, 28% of loon clutches have only one egg (McIntyre, 1988). Single egg analysis for Hg thus represents a potentially confounding factor that could influence future study design and interpretation.

Over time, body burdens of Hg are known to significantly increase in adult loons in response to elevated levels of dietary MeHg. Although blood-Hg concentrations may not demonstrate significant increases over the lifetime of the same individual, feather Hg concentrations do tend to increase with age (Evers et al., 1998). In New England, feather Hg concentrations significantly increased at a rate of about 5.7% annually for recaptured female loons (Evers et al., 2002). Hg in feathers is predominantly in the MeHg form, and correlates with MeHg concentrations in muscle. Muscle tissue provides the protein used during feather formation (Crewther et al., 1965) and is one of the few body compartments from which MeHg can be remobilized. Tapping into this MeHg reserve during molt serves as an important MeHg depuration route for birds (Braune and Gaskin, 1987); and feather-Hg concentration can thus be used as an indicator of MeHg body burden during feather growth in birds (Burger, 1993).

However, there are three lines of evidence that indicate that, unlike the situation for feathers, MeHg in muscle provides only minor contributions to Hg in the developing egg. First, as we have shown in this study, egg-Hg concentrations are strongly correlated with female blood-Hg concentrations, which closely reflect prey Hg concentrations on the breeding lake (Burgess et al., 1998a; Evers et al., 2002). Second, female feather-Hg concentrations do not correlate with

egg-Hg concentrations ($F = 1.24$, $df = 257$, $p > 0.05$; Biodiversity Research Institute (BRI), unpubl. data). Last, because dietary MeHg exposure on the loon's marine wintering areas is generally low [adult loons captured from coastal Maine south to Florida had mean blood-Hg concentrations of $0.47 \pm 0.23 \mu\text{g/g}$ with no samples exceeding $1.0 \mu\text{g/g}$ ($n = 63$, BRI unpubl. data)] compared to that on their fresh water breeding lakes, the contribution to Hg in loon eggs from MeHg stored in muscle or other tissues is probably minor compared with contributions arising from the diet that is being consumed during egg development.

Although Hg depuration through eggs is substantial in female birds, relationships between eligible body burdens of MeHg in key tissues, such as muscle, and Hg deposition into eggs, are generally weak (Lewis et al., 1993; Bearhop et al., 2000). Although exceptions are known, such as the osprey (*Pandion haliaetus*) (Hughes et al., 1997) we have found that, in the common loon, egg-Hg concentrations primarily reflected MeHg concentrations of major prey items from the lake on which the female loon feeds and nests (Evers et al., 1998; Scheuhammer et al., 1998b). However, loons that maintain multi-lake territories will tend to increase the variability of egg-Hg vs. blood-Hg relationships. Loon pairs nesting on water bodies <24 ha generally incorporate more than one lake in their territory (Piper et al., 1997b); in such circumstances, adult loons may use lakes neighboring the natal lake for foraging purposes. Because MeHg production and availability can vary substantially among lakes that are in close proximity, or even within the same watershed, blood-Hg levels for more mobile loons may not accurately represent the dietary uptake of MeHg from the natal lake.

Reproductive effects of Hg

Controlled studies have shown that Hg negatively affects embryonic development and hatchability of avian eggs at levels that are found in some eggs in the current study (Fimreite, 1971; Gilbertson, 1974; Heinz, 1979). Thompson (1996) summarized several controlled dosing studies of captive birds and concluded that egg-Hg concentrations of $0.50 \mu\text{g/g}$ (ww) may cause impairment in some bird species. Barr (1986) found loons laid fewer eggs when prey-Hg concentrations averaged $0.3\text{--}0.4 \mu\text{g/g}$, and egg-Hg concentrations exceeded $0.60 \mu\text{g/g}$; no eggs were laid when prey averaged over $0.4 \mu\text{g/g}$ of Hg. Several of

our study lakes contained preferred prey and size classes (yellow perch, *Perca flavescens*, between 10 and 15 cm) (Barr, 1996) for which Hg concentrations exceeded 0.3 and $0.4 \mu\text{g/g}$ (Evers et al., 2002).

Unlike controlled dosing studies that showed a reduction in egg fertility as egg-Hg concentrations increased (Fimreite, 1971; Thaxton and Parkhurst, 1973), we did not find a significant relationship between egg-Hg concentrations and percent of infertile eggs (Table 4). However, we collected only abandoned eggs; a random sample of eggs is needed before the potential impacts of Hg on loon egg fertility can be properly assessed. In the current study, Hg concentrations in loon eggs did not significantly differ among five developmental stage categories ($F = 1.41$, $p = 0.23$). But because we frequently collected only one egg from 2-egg clutches, and did not explicitly investigate causes of hatching failure, further investigation of this issue is warranted.

We found that intraclutch variability in egg-Hg concentrations increased as Hg concentrations increased. It is likely that, for laying females with higher blood-Hg concentrations, there will be a greater difference in the Hg concentrations of the two eggs laid. Depending on a study's objectives, this confounding factor further underlines the need to analyze both eggs in a clutch, particularly in situations where MeHg availability is high.

A relationship between smaller egg size and higher Hg concentrations has been shown previously in a controlled study (Fimreite, 1971). Similarly, in the current study, we found that egg volume significantly decreased as Hg concentration increased. The egg-Hg risk category of " $>1.3 \mu\text{g/g}$ " was comprised of eggs significantly smaller than in the Hg risk category of " $0\text{--}0.6 \mu\text{g/g}$ " ($t = 2.14$, $p = 0.03$). Independent of Hg toxicity, this finding is contrary to previous studies that reported that the first loon egg laid is the largest (Yonge, 1981; McIntyre, 1988); and that, in birds generally, the first egg laid has higher Hg concentrations than subsequent eggs (Becker and Sperveslage, 1989; Becker, 1992). But because, in loons, egg size (measured as fresh egg weight) is strongly related to female body weight ($r^2 = 0.82$, based on $n = 613$ eggs and 497 female weights, BRI unpubl. data), and because a lower female body weight reduces reproductive success (in ducks, Blums et al., 1997), our findings may indicate a decreased physiological condition of female loons in habitats with high risk for elevated Hg exposure.

Establishing Hg risk levels for loon eggs

The many studies investigating Hg concentrations in avian eggs and associated effects provide a basis for estimating risk levels for the common loon (Thompson, 1996; Scheuhammer et al., 2001). We have identified four categories of risk based on Hg in loon eggs (Table 4).

The first category of 0–0.60 µg/g (low risk) indicates background Hg concentrations that reflect (1) natural environments that do not experience enhanced local geological sources of Hg or inputs from natural Hg sources, such as volcanic activities, nor from anthropogenic deposition; and (2) environments that may receive enhanced Hg inputs (either natural or anthropogenic), but which lack local conditions that favor MeHg production and availability. Evidence from the literature indicates no adverse impacts to birds at egg-Hg concentrations <0.50 µg/g (Thompson, 1996). Because Barr (1986) found reproductive impairment in loons when Hg concentrations exceeded 0.60 µg/g we have used this value as our best estimate of a lowest observed adverse effect level. The second category of 0.60–1.30 µg/g (moderate risk) reflects elevated levels of environmental Hg that may be indicative of significant reproductive impairment in some individuals of some avian species, including loons. A third category of >1.3 µg/g (high risk) is indicative of an environment where loons are at high risk for reproductive impairment. Adult female loons with blood-Hg concentrations >3.0 µg/g, laid eggs containing >1.30 µg/g (Fig. 2), and often had decreased reproductive success, laying significantly fewer eggs, which in turn demonstrated decreased hatching success (Evers et al., 2002). Furthermore, egg size was significantly lower for eggs with Hg concentrations >1.30 µg/g compared to eggs in low and moderate risk categories. Similarly, concentrations >1.3 µg/g were suspected to result in reduced hatching success in a field study with common terns (Fimreite, 1974) and were associated with reduced hatchling survival in a controlled lab study with mallards (Heinz, 1976a,b). Finally, egg-Hg concentrations exceeding the 2.0 µg/g (ww) (Xhigh risk) are widely considered to have detrimental reproductive effects in most bird species (Thompson, 1996).

Regional rating of Hg risk

Based on our findings and on comparisons with the results of other studies, the four egg-Hg risk categories

provide our best estimate for interpreting egg-Hg concentrations in common loons found across North America (Fig. 3). Using these categories, a regional comparison indicates low Hg risk for common loons in Alaska, Montana and much of the Canadian Prairie Provinces. Fox et al. (1980) found low Hg risk on Hanson Lake in east-central Saskatchewan where 31 eggs had Hg concentrations ranging from 0.13 to 0.75 µg/g.

The western Great Lakes region is characterized by low to moderate Hg risk, although there are well known hotspots in north-central Wisconsin (Meyer et al., 1998; Fevold et al., 2003) and western Upper Peninsula of Michigan (Evers et al., 1998) related to the prevalence of acidic lakes; and in north-western Ontario related to a chlor-alkali plant (Parks and Hamilton, 1987). In this latter area, Barr (1986) found Hg concentrations in loon eggs from the mid 1970s ranging from 0.54 to 1.39 µg/g ($n = 34$), and documented reduced reproductive success. Belant and Anderson (1990) found egg-Hg concentrations ranged from 0.40 to 1.10 on the Turtle–Flambeau Flowage in north-central Wisconsin (mean of 0.64 ± 0.21 , $n = 16$).

The majority of loon eggs collected in the eastern Great Lakes region have Hg concentrations indicative of low to moderate risk, but compared with the western Great Lakes region, there appears to be a somewhat greater risk posed to more eastern loons, with some lakes exhibiting high (Vermont) and extra high Hg risks (eastern Ontario). Again, this may be due primarily to a prevalence of low pH/low alkalinity environments. Between 1969 and 1980, Frank et al. (1983) collected eggs in the Algonquin-Parry Sound area and found mean Hg concentrations ranging from 0.81 to 1.11 µg/g, with some eggs exceeding 1.40 µg/g, indicating that some high risk lakes existed during that time period. Scheuhammer and Blancher (1984) estimated that 30% of lakes in eastern and central Ontario contained small fish with Hg concentrations sufficiently high to pose a risk to loon reproduction. Although the eggs we collected in New York did not indicate high Hg risk, some reservoirs (McIntyre et al., 1993) and low pH lakes (Simonin et al., 1994) are known to contain fish with Hg concentrations that place loons at high risk. Eggs collected from New York's Stillwater Reservoir between 1978 and 1986 ($n = 25$) had a mean Hg level of 1.6 ± 0.6 µg/g with 64% of the eggs having > 1.3 µg/g and 28% having >2.0 µg/g (Holmquist, 1990).

Loon eggs from northeastern North America are generally in higher risk categories than those from other areas of North America, with some areas in Maine, New Hampshire, and Nova Scotia containing loon eggs that are likely not hatching because of environmental MeHg transfer (i.e., $>2.0 \mu\text{g/g}$). Certain geographic Hg hotspots are now well known in southeastern New Hampshire, the western mountains of Maine, near a chlor-alkali plant in Downeast Maine (now closed), and in Kejimikujik National Park, Nova Scotia. Investigations into potential population-level impacts of Hg on loons breeding in northeastern North America are ongoing (Burgess et al., 1998b; Evers et al., 2002)

Wildlife conservation and Hg

Developing regulatory policies for reducing Hg emissions and effluents are difficult, but high priority, conservation issues. Policy-makers at national (USEPA, 1997) and regional (NESCAUM, 1998) levels have compiled and assessed the available ecological and human health concerns and research. Landscape-level investigations into the distribution patterns of Hg have been made possible by programs such as the US Department of Agriculture's Northeastern Ecosystem Research Cooperative. However, while our and other studies on Hg in common loons are contributing valuable new scientific information, there will ultimately remain a need for more ecologically relevant and comprehensive approaches (Peakall and Tucker, 1985). Indications of ecological risk in northeastern North America from this and other related loon-Hg studies underscore a pressing need for broader investigations.

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