

North American Common Loon Biomonitoring Program

Quebec, Canada

1997-01 Comprehensive Report

(BRI 2003-01)



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(Report BRI2003-01)



Submitted to:

**Canadian Wildlife Service
Canadian National Park Service**

By

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BioDiversity Research Institute is a Maine-based nonprofit research group dedicated to progressive environmental research and education that furthers global sustainability and conservation policies.



Fundamental studies involve avian conservation and aquatic toxicology. We believe high trophic level piscivorous wildlife are vital indicators of aquatic integrity.

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Background

Mercury (Hg) is a naturally occurring element in our environment and is prevalent in aquatic ecosystems. However, analysis of sediment cores from lakebeds indicates that current rates of Hg deposition in mid-continental North America are 3-4 times greater than pre-industrial levels (Swain et al. 1992). Moreover, Hg has been demonstrated to affect the behavior, reproduction, and survival of wildlife and humans (Eisler 1987, Hoffman and Moore 1979, Thompson 1996, Wiener and Spry 1996). Anthropogenic sources (such as coal burning and incinerator emissions) deposit Hg over our landscape via wet and dry atmospheric particulate fallout. Studies comparing fish Hg concentrations with rates of atmospheric deposition have found that these sources account for much of the Hg loading into aquatic ecosystems (Fitzgerald 1995, Rada et al. 1989, Rudd 1995).

Nearly all mercury (>95%) that circulates in the atmosphere occurs in the elemental form (Hg^0) (Nader and Grigal 1992). Bacteria in aquatic ecosystems convert inorganic mercury into a more toxic organic form called methylmercury (MeHg). Once MeHg is available in the food chain it is concentrated, or biomagnified, in higher trophic-level animals.

While mercury is a potent neurotoxin, determining its effects on wildlife is difficult. The dynamics of mercury cycling in the environment are complex (Watras and Huckabee 1994). Concern over Hg levels is increasing because of the potentially small margin of safety between background levels of exposure and concentrations that can harm humans and other organisms (Michigan Environmental Science Board 1993). A challenge to researchers studying Hg in the biotic system is identifying the threshold at which wildlife is impacted by Hg exposure.

Aquatic predators such as the Common Loon (*Gavia immer*) are useful indicators of mercury in the environment (Burgess et al. 1998, Evers et al. 1998, 2003, Meyer et al. 1995). The loon's prominence as Canada's national bird and icon of wilderness makes it a popular species with the public. Governmental and public support facilitates long-term studies that can determine the scope and magnitude of environmental contaminants on important wildlife populations.

Study area

BioDiversity Research Institute (BRI), a non-profit ecological research group, with collaboration across North America, has developed the North American Loon Biomonitoring Program as means to collect and disseminate information on behavior, population ecology, and contaminant exposure in loons. Study sites are distributed across a large geographic area, providing opportunities for regional and site-specific comparisons.

In cooperation with the Canadian Wildlife Service, North American Loon Fund, and the Canadian National Park Service, biologists from BioDiversity Research Institute sampled Common Loons from two sites in Quebec in the summers of 1997 and 1998, four sites in August 1999, and three sites in 2001. In 1997, sites included La Mauricie National Park (LMNP) in eastern Quebec and 31 Mile and Pemichangan Lakes in the Gatineau region of the province. Sampling in 1998 was limited to LMNP and a new site: St-Mauricie Provincial Reserve (SMPR). LMNP is located 135 km west of Quebec City and 135 km



northeast of Montreal and SMPR abuts the LMNP to the north. In 1999 our sampling efforts included 2 lakes in the Gatineau region of Quebec: 31 Mile lake and Pemichangan, 3 lakes in LMNP, lakes in Laurentides Region: 3 lakes in Parc du Mont Tremblant and 2 lakes outside of the park, and Lac des Iles in the Shawinigan Region. The lakes sampled in the Gatineau region in 1997 and 1999 are located 90 km north of Ottawa City and just east of the Gatineau River. In 2001, we captured and banded loons again in LMNP and added two new lakes in Laurentides Region and two lakes in Quebec Region (Figure 1).

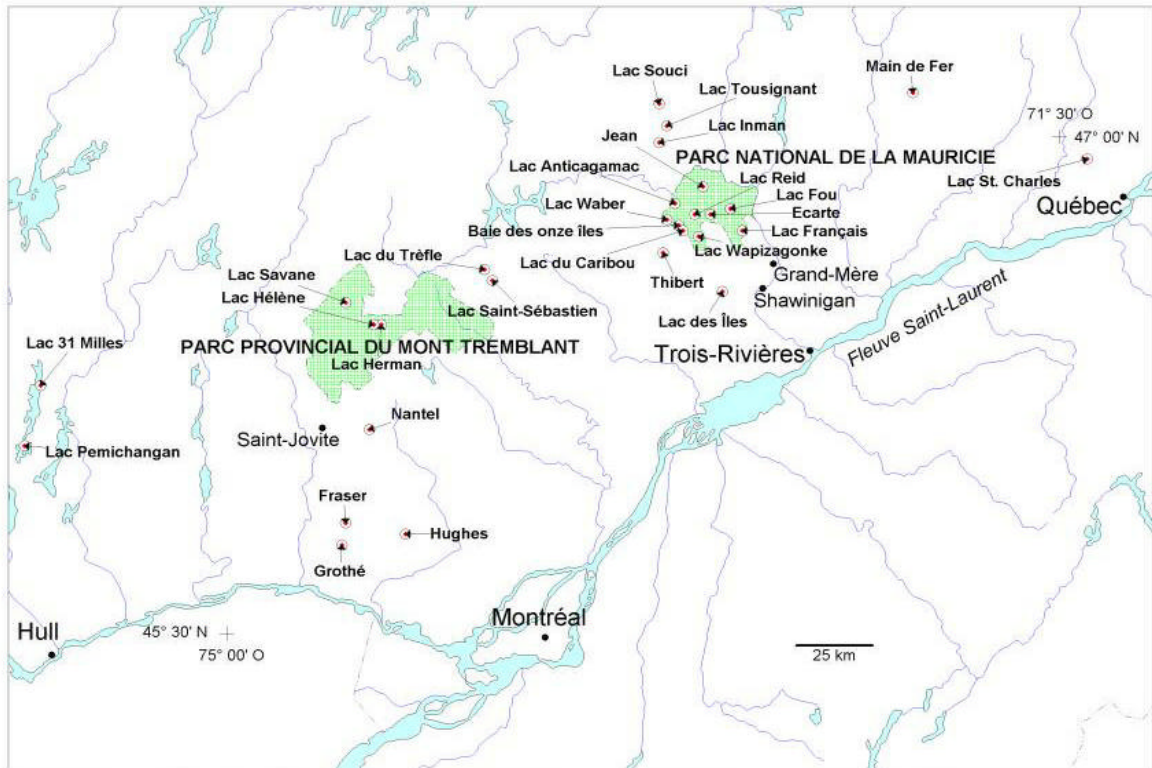


Figure 1. Common Loon study sites and lakes sampled, Quebec 1997-01.

In addition to banding and sampling efforts, detailed productivity information on breeding loons has been collected at LMNP since 1987 by park biologist Denis Masse. The effects of Hg on loon productivity should be examined when sample sizes are sufficient. A similar approach in New Brunswick and Nova Scotia is proving useful in assessing the relationship between loon productivity and mercury exposure (Burgess et al. 1998).



Methods

Sample Collection

Loons were captured from canoes and small motorboats using the night lighting capture technique developed by BioDiversity Research Institute (Evers 2001). A million-candlepower spotlight, tape recordings and mimicked vocalizations of loon calls attracted family groups to the boat. Individual loons were caught with a large landing net, restrained, and brought to shore where information and samples were collected.

Both second secondary flight feathers were removed from adult birds by cutting at the calamus (below the base of the feather tract on the vein). Blood samples were taken from the medial metatarsal vein with 20-25 gauge butterfly needles fitted with multiple sample Luer Adapter into 7 cc Vacutainers® containing powdered sodium heparin (green top), 5 cc Vacutainers® containing calcium EDTA (purple top), and 5 cc Vacutainers® containing no additive (red top). Both blood and feather samples were later split between the Canadian Wildlife Service and BioDiversity Research Institute for comparative analysis of Hg. Blood samples were also collected by Tufts University School of Veterinary Medicine for immunological and genetic analyses. 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 per 1 cc whole blood) (Stafford and Stickel 1981, Wiemeyer et al. 1984). All loons were uniquely marked with an aluminum USFWS band and 1-3 colored leg bands glued with an acetone-based adhesive. Finally, each individual was weighed prior to being released unharmed in its respective territory. Each family was monitored to ensure that adults and juveniles regrouped after capture.

Sample Analysis

One feather from each adult and a blood sample from each individual loon were analyzed for Hg concentrations at the Animal Health Diagnostic Laboratory at Michigan State University in 1997 and the University of Pennsylvania in 1998, 1999 and 2001 following identical protocols. Feathers were cut (calamus discarded) and washed three times in acetone (chromatography grade, Burdick and Jackson, Muskegon, MI), three times in ultra pure water (4 bowl MilliQ System, Millipore Corp, Bedford, MA), one additional time in acetone, and then placed in a fume hood to dry overnight. Hg binds strongly to disulfide linkages of keratin in feathers (Crewther et al. 1965) and is not disturbed by washing episodes (Applequist et al. 1984) or previous environmental exposure (Goede and de Bruin 1984). A 0.25-0.50g aliquot of each feather was digested overnight at 90° C with 2 ml conc. HNO₃ (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 10 ml volumetric flask and diluted an additional 1000 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 $0.27 \pm 0.06 \mu\text{g/g}$ (National Research Council of Canada, Ottawa, Canada). Each sample was finally analyzed for trace metal and mineral content again using ICP emission spectroscopy.

A 100 mg 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 25 ml (chicks) or 100 ml (adults) volumetric flasks, diluted to volume with ultra pure water, and analyzed as above by cold-vapor atomic absorption spectroscopy.

Results and Discussion

Between 1997 and 2001, we captured 58 adult (three of which were recaptures) and 27 juvenile common loons in the province of Quebec (Table 1). Eighteen of the adults were banded in the Gatineau Region (Western Quebec) (Table 2) and the remaining 40 in the Eastern Region (La Mauricie, Laurentides, and Quebec) of the province. Of 27 juvenile loons captured one was from Gatineau and 26 from the eastern region.

In 1997 and 1998, a total of 34 Common Loons from 15 territories were captured in Quebec. Twenty three loons were captured in Mauricie region, and 11 adult loons were captured at the Gatineau sites (Tables 1, 2). In addition, four Common Mergansers were captured in SMPR on Lac Brown. In La Mauricie area, 13 loons were captured in 1997 and 10 loons in 1998.

In 1999 31 loons from 11 lakes were captured in Quebec: 7 adults (1 was recaptured from 1997) and 1 juvenile in Gatineau region, 8 adults and 3 chicks in Tremblant region (Laurentides), 4 adults (1 recapture) and 5 chicks were caught in LMNP, and 2 adults and 1 chick from Lac des Iles in Shawinigan region.

In 2000, we did not conduct fieldwork in the province.

In 2001 we captured 20 loons from seven lakes in Quebec: 5 adults and 6 juveniles from LMNP, 3 adults and 2 juveniles from Laurentides region, and 2 adults and 1 juveniles from Quebec Region.



Table 1. Mercury concentrations in Common Loons captured in several regions of Quebec, 1997-2001 (loons were not sampled in 2000).

Territory	Blood Hg ($\mu\text{g/g}$, ww)				Feather Hg ($\mu\text{g/g}$, fw)			Weight (g)			
	Male	Female	Juvenile1	Juv. 2	Male	Female	Juvenile	Male	Female	Juvenile1	Juv. 2
La Mauricie Region											
<i>La Mauricie NP (LMNP)</i>											
Anticagamac	1.10	1.40			8.40	8.13		4550	4300		
Baie de Onze Iles		0.81				5.01			4000		
Caribou-South-1997	3.90	1.10	0.013		48.60	7.27		5200	3750	1700	
Caribou-South-1998			0.025							1300	
Caribou-South-1999 ¹	3.58				78.20			5400			
Caribou-beach ²	0.79				8.28						
Ecarte	2.85		0.321		30.70			4800		2150	
Fou (Du)											
East-97	1.50	2.10	0.320		17.00	8.60		4000	3475	1450	
East-99	3.62		0.118		14.00			4475		1650	
East-01 ³	4.38		0.551		23.00			4725		935	
West-97			0.410	0.46						1550	1950
West-99			0.072							1350	
Francais	2.60				17.00			4225			
Reid	2.40				15.20			4950			
Waber	4.00				24.40			5250			
Wapizagonke-Basin 1, 1998	2.33	0.68			14.70	5.79		4000	3400		
Wapizagonke-Basin 1, 1999			0.104							750	
Wapizagonke-Basin 1, 2001			0.383							2225	
Wapizagonke-Basin 2, 2001 ⁴	1.57		0.260		17.80			4575	3350	1450	
Wapizagonke-Basin 3, 1999	2.32	1.64	0.069		12.60	7.90		5750	3675	1175	
Wapizagonke-Basin 3, 2001		1.42	0.255			17.70				400	
Mean (LMNP)	2.64	1.31	0.240		23.56	8.63		4762	3707	1431	
<i>La Mauricie (other lakes)</i>											
Lac Inman		2.23				9.75			3900		
Souci		1.79	0.013			13.10			3850	1850	
Tousignant	3.17		0.013		16.90			4140		710	
Thibert	2.21		0.376		11.10			5075		2725	
Mean (other lakes)	2.69	2.01	0.134		14.00	11.43		4608	3875	1762	
Mean La Mauricie Region	2.64	1.46	0.221		22.37	9.25		4741	3744	1489	
Shawinigan-Lac Des Iles-1998 ⁵			0.848				25.7	5200	4700	3000	
Shawinigan-Lac Des Iles-1999	8.63	7.44	1.130		19.40	7.80				3700	
Laurentides Region											
Herman		0.51	<0.025			6.60			3325	1825	
Hughes	0.90	0.95	0.250		13.40	9.16		4970	3700	2600	
Helene	5.50	5.80	0.300		23.40	15.80		4750	3825	1850	
Savane	3.10				16.80			5475			
Du Trefle-South	1.19	0.74			9.05	14.50		4525	3250		
Du Trefle-West		0.10				4.47			3825		
St.Sebastien	1.75		0.096		9.06			4300		1200	
Mean (Laurentides)	2.49	1.62	0.215		14.34	10.11		4804	3585	1869	
Quebec Region											
Main de Fer			0.487	0.703						2350	2575
St. Charles-South	1.60	1.25	0.250		12.90	6.46		5325	4550	2975	
St. Charles-North	1.78				7.17			4925			
Mean Quebec Region	1.69	1.25	0.480		10.04	6.46		5125	4550	2633	
Mean +/-stdev	2.53+/-1.2	1.5+/-1.3	0.25+/-0.2		19.6+/-15.6	9.4+/-4.1		4790+/-493	3745+/-363	1696+/-685	

¹ Captured in 1997 and 1999² This bird was found dying from lead poisoning and was emaciated, his weight was 2650 g³ Captured in 1999 and 2001⁴ recapture; the male was captured in 1998 in Basin 1⁵ This lake is an outlier because of extremely high blood Hg conc. in loon blood and is left out of all calculations of the means.

Adult Blood and Feather Hg Exposure

In Lac des Iles (Shawinigan) adult (male=8.63 ppm, female=7.44 ppm) and juvenile mean (0.99 ppm) blood Hg concentrations are doubled those in other sites in Quebec, therefore we exclude this lake from all calculations and statistical analyses, and report it separately. The birds from this lake are among the heaviest loons we measured in Quebec. It could be one explanation for higher Hg concentration as they consume larger fish with higher Hg levels.

The mean blood Hg concentrations in adults in eastern Quebec were about 70% higher (male 2.53+/- 1.12 µg/g, and female 1.5+/-1.33) than in the western Quebec (Gatineau region) (male 0.74 +/- 0.29 µg/g, and female 0.50+/-0.24). The mean feather Hg concentration in adult male loons from eastern Quebec regions (19.6+/-15.6 µg/g) was 49% higher than the Gatineau sites (10.0 +/- 3.5 µg/g), and mean feather Hg concentrations in females were 33% higher (9.3+/-4.1 vs. 6.2+/-2.3 in Gatineau). Compared to other sites in North America, loons in Gatineau had low blood Hg levels (for both sexes): similar to Alaska concentrations (Figure 2).

Mean whole blood Hg concentrations in adult male Common Loons were similar between La Mauricie (2.64 µg/g) and Laurentides (2.49 µg/g) regions. The mean male blood Hg from the Quebec region was lower (1.69 µg/g) but only two males were sampled. Blood Hg levels in males at La Mauricie region ranked fourth highest among the North American sites: they were lower compared to the Canadian Maritimes, Maine, and the Laurentides region (Figure 2). Female blood Hg levels at LMNP were lower than in their counterparts from Canadian Maritimes, Ontario, Maine and Laurentides region.



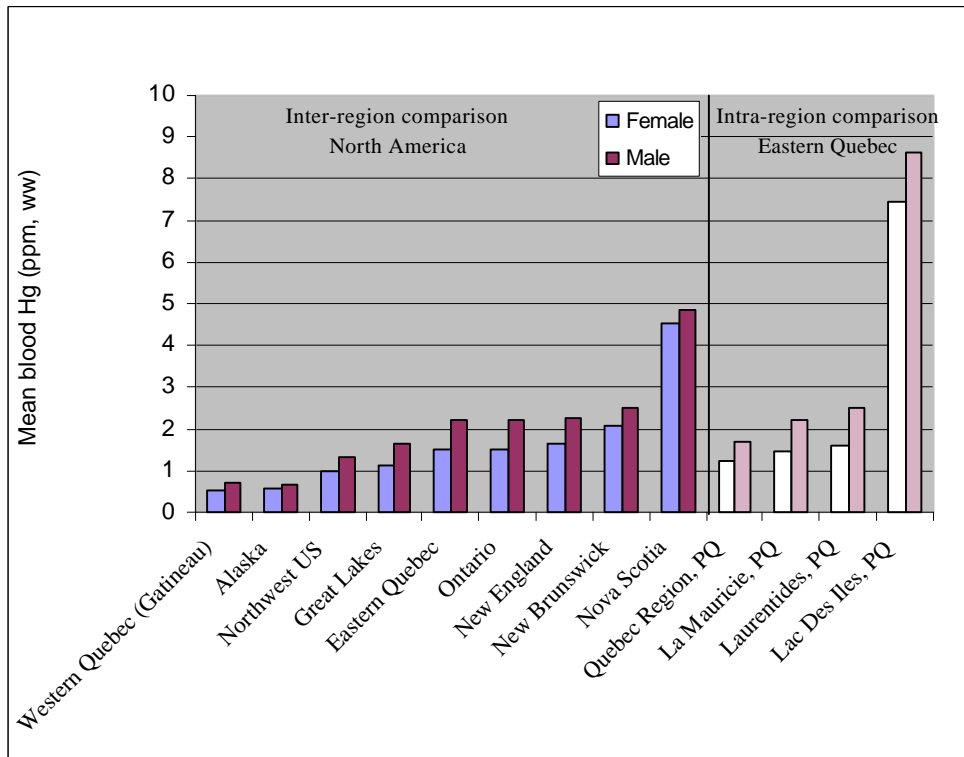
Table 2. Mercury concentrations in Common Loons from the Gatineau Region, Quebec, 1997-1999.

Territory	Blood Hg (µg/g)			Feather (i g/g)		Weight (g)	
	Male	Female	Juvenile	Male	Female	Male	Female
<i>31 Mile Lake</i>							
Bear Creek Bay*	0.70		<0.025	8.3		5225	
Hay Island	0.46	0.46		5.0	8.0	5475	4050
Green Bay	1.00	0.50		11.3	5.4	5125	3950
Sand Bay	1.10	0.74		11.4	9.9	4900	3800
Weber Cove	0.62			6.0		5100	
Big Loge Bay	0.45	0.20		11.4	4.4	5600	3880
Grande Ile	1.05			10.6		4800	
Long Island	0.40	0.17		9.3	4.9	5010	3640
<i>Lake Pemichangan</i>							
Chantigny Bay		0.63			7.6		3800
Ritchie Bay	0.83	0.79	0.6**	17.0	3.3	4875	4325
Mean +/-stdev (n)	.73+/-0.27	0.50+/-0.24	<0.025	10.0+/-3.5	6.2+/-2.3	5123+/-272 (9)	3921+/-220 (7)

* This loon was recaptured in 1999, blood Hg=0.32 ppm, feather Hg=8.31ppm

** Adult, sex unknown

Figure 2. Common Loon mean blood Hg concentrations at selected sites in North America



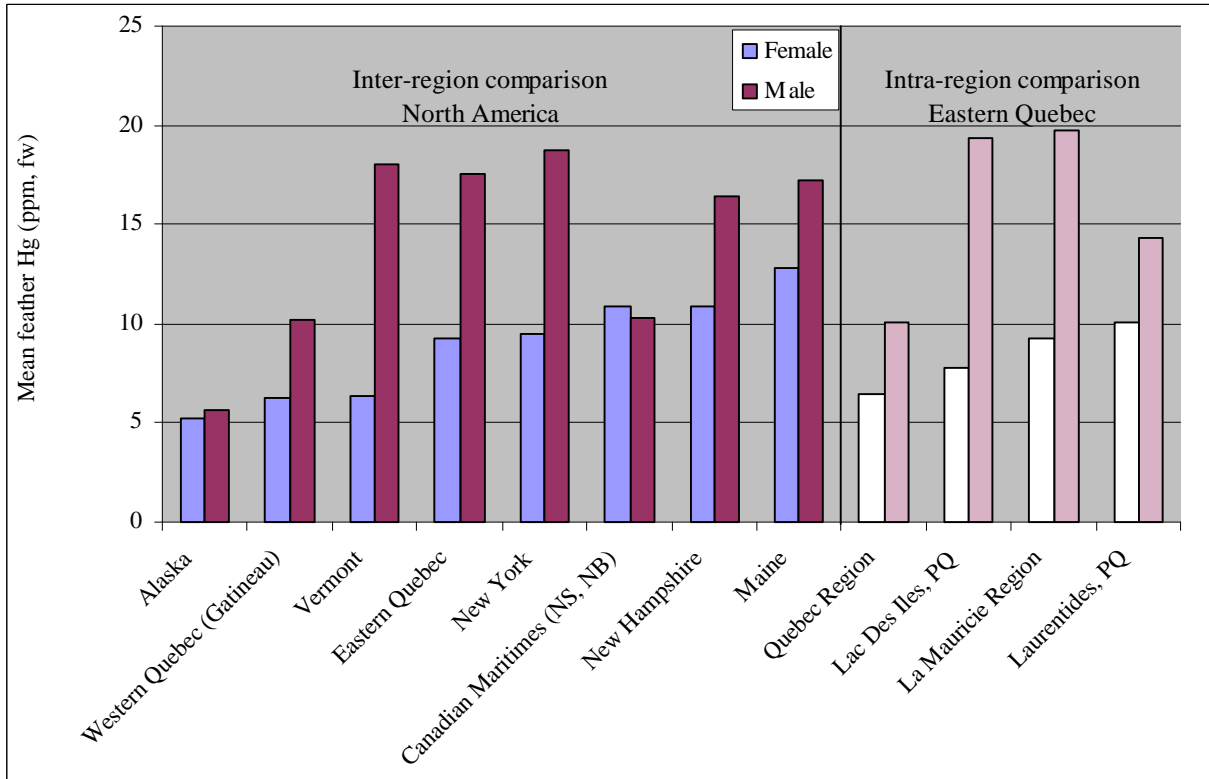
Adult male loons typically have higher Hg levels than females in both blood and feathers (Evers et al. 1998). The mean difference between North American male and female loons is 21% in blood (2-46%) and 26% in feathers (6-35%). At the Gatineau site, mean Hg concentrations were 32% and 38% higher in male than female blood and feathers, respectively. At LMNP, male loons had 39% higher Hg in the blood and 58% more Hg in the feathers than females. At SMPR mean blood mercury concentrations in males were 36% higher and feather Hg was 33% higher than in females. In Tremblant lakes male blood Hg levels were 38% higher and feathers were 29% higher than in females. Combining all values from all the loons sampled in eastern Quebec, mean blood and feather Hg levels in males were 33% and 47% (respectively) higher than in female loons. The differences in Hg concentrations between sexes could be attributed to the different prey selection by the two sexes. Male Common Loons are bigger than the females therefore select and consume bigger fish (with subsequently higher Hg concentrations) (Evers et al. 2002). Females are able to depurate some of their Hg through the eggs, but it does not significantly account for differences in Hg levels between genders (Evers et al. 2003).

The large difference in mean feather Hg concentrations between male and female loons at LMNP can be partially explained by the mean feather Hg level of 63.4 µg/g in the Lac Caribou male (almost 2 times greater than any other LMNP loon) (Table 1). This is the highest known feather Hg of any loon tested in North America. In 1999, we recaptured this male in Lac Caribou, his feather Hg measured 78.2 µg/g. Despite the extremely high Hg levels this bird reproduced successfully. It is likely an old bird, and therefore had many years to bioaccumulate Hg in its body. Previously, the highest North American feather Hg concentration measured in a loon captured in Ottawa National Forest, Michigan. A male loon at that site had 40.6 µg/g Hg in the feathers and was unable or unwilling to incubate its eggs, which subsequently failed to hatch. Later in the season, the loon was recovered dead, apparently killed by another loon (puncture wounds were noted in the sternum). A subsequent necropsy found >200 ppm of Hg in its liver and cause of death was diagnosed as Hg poisoning.

On average, feather Hg concentrations in male loons from La Mauricie National Park, La Mauricie Reserve, Laurentides, and Lac des Iles are similar and comparable to the mean of the Northeast but are elevated in comparison with control sites in the Northwestern United States and Alaska. (Figure 3). Several male loons from certain lakes have levels (>20ppm Hg) that potentially indicate high chronic Hg exposure (Table 1). Females from La Mauricie, Laurentides, and adults from the Gatineau region had Hg levels similar to other sites in North America.



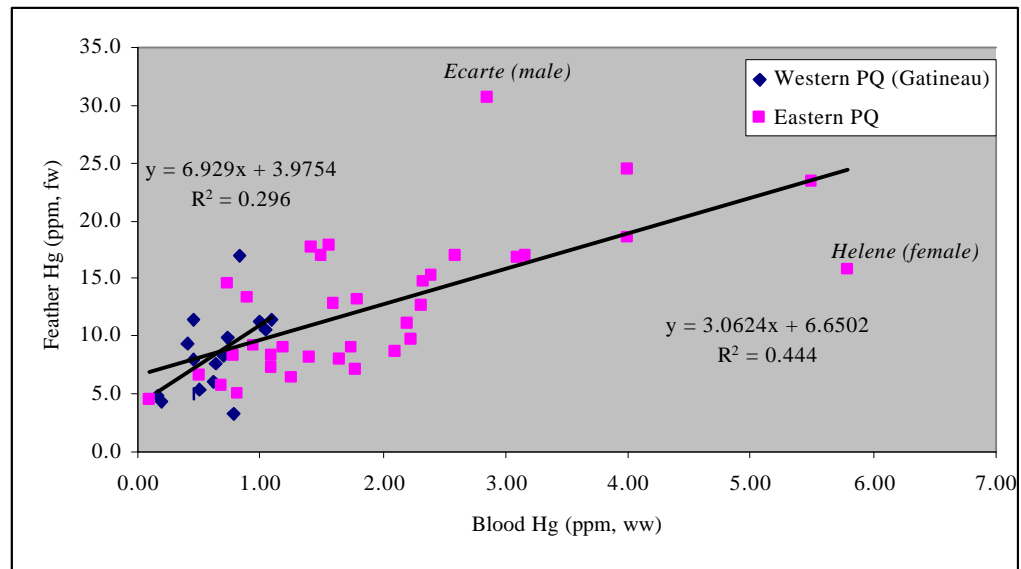
Figure 3. Adult Common Loon mean feather Hg from selected sites in North America.



Adult whole blood and feather Hg concentrations across North America show a highly significant positive relationship ($F=55.4$, $p<0.001$), however the correlation coefficient is weak ($r^2=0.20$). The significance of the blood-feather relationship increases from west to east. The weakest correlation is in Alaska ($r^2=0.03$) while the Canadian Maritimes have the highest correlation ($r^2=0.48$) for this relationship (Evers et al. 1998). Feather Hg, which is sequestered in the winter during a full remigial molt, is therefore directly related to breeding ground Hg ingestion at site with high Hg concentrations in the prey. Loons breeding on lakes with low Hg levels do not bioaccumulate high amounts of Hg and therefore do not have extra Hg to flush through their feathers. Consequently, feather Hg in loons on low Hg lakes does not increase with bird's age. In the eastern regions of Quebec (La Mauricie, Laurentides and Quebec), there is a stronger correlation between blood and feather Hg levels ($r^2=0.44$) (the Caribou male and Lac des Iles birds are excluded), than in the Gatineau region where the correlation is weaker ($r^2=0.30$) (Figure 4).



Figure 4. Relationship between blood and feather Hg in adult Common Loons, Quebec 1997-01. (excluding Lac des Iles and one Caribou male Hg values)



Juvenile Hg Exposure

Blood Hg concentration in juveniles is an excellent indicator of local Hg availability, since loon chicks are fed exclusively from the natal territory. The chicks large enough to sample in LMNP had high blood Hg burdens for their age (Table 3). Two chicks captured in SMPR had low mercury concentration (0.0125 ug/g). The chicks from Lac des Iles in Shawinigan Region had the highest blood and feathers mercury levels in Quebec. The chick captured in 1998 had the highest feather Hg level we have recorded in any juvenile (25.7 ug/g). Only one chick was caught in Gatineau region, its blood Hg concentration was below instrument detection limit of 0.025 ppm.



Table 3. Mean blood mercury concentrations in juvenile Common Loons at selected sites in North America, 1992-2001*

Location	Blood Hg Values ($\mu\text{g/g}$)		
	Mean +/- SD (n)	Range	Blood Index [^]
Province of Quebec**	0.25 +/- 0.20 (24)	0.01-0.70	1.22 +/- 0.77
<i>Shawinigan-Lac des Iles</i>	0.99 +/- 0.21 (2)	0.85-1.15	3.03 +/- 0.04
<i>Quebec Region</i>	0.48 +/- 0.23 (3)	0.25-0.703	1.88 +/- 0.96
<i>La Mauricie National Park</i>	0.24 +/- 0.15 (14)	0.01-0.55	1.37 +/- 0.83
<i>La Mauricie Other Lakes</i>	0.13 +/- 0.20 (3)	0.01-0.38	0.122 +/- 0.08
<i>Laurentides Region</i>	0.22 +/- 0.11 (4)	<0.025-0.3	1.15 +/- 0.44
<i>Gatineau Region</i>	<0.025 (1)	-	-
Canadian Maritimes (NB, NS)	0.35 +/- 0.16 (10)	0.12-0.57	
Northeast (ME, NH, NY, VT, PQ)	0.20 +/- 0.34 (104)	0.01-2.14	0.89 +/- 1.27
<i>Maine</i>	0.29 +/- 0.43 (54)	0.01-2.14	1.30 +/- 1.53
<i>New Hampshire</i>	0.10 +/- 0.12 (33)	0.01-0.42	0.46 +/- 0.58
<i>Vermont</i>	0.01 +/- 0 (2)	-	0.05 +/- 0.01
<i>New York</i>	0.02 +/- 0.02 (10)	0.01-0.06	0.01 +/- 0.05
Great Lakes (MI, MN, ON, WI)	0.14 +/- 0.11 (142)	0.04-0.61	
Northwest (WA, MT)	0.10 +/- 0.05 (6)	0.09-0.19	
Alaska	0.07 +/- 0.06 (14)	0.02-0.27	

* Data from Quebec collected 1997-01; all other data collected 1992-1998.

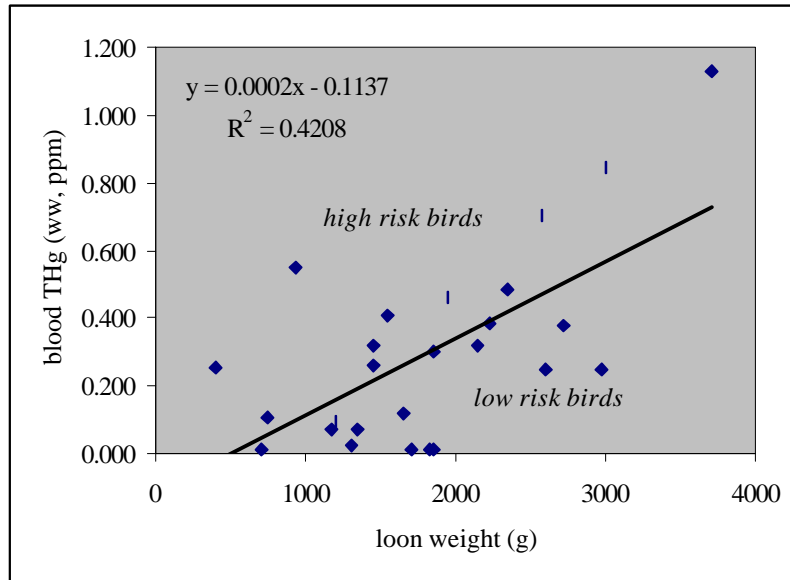
[^] Index calculated as follows: {individual blood Hg (ppm)/body weight (g) x 10,000} = (ppmHg/g) x 10⁴

** Quebec means exclude Lac des Iles and Gatineau region data.

In Maine, Evers and Reaman (1997) found that a significant increase in blood and feather Hg levels during the juvenile loon's first 12 weeks was most apparent on lakes where small fish (10-15 cm) had Hg concentrations greater than 0.30 ppm ($r^2=0.78$, $p<0.05$). Therefore, juvenile loons from contaminated lakes are likely to accumulate Hg and need to have their blood Hg levels adjusted by their weight (which correlates with age) to provide a standard index of Hg exposure (Figure 5). Based on our loon work in Maine, we estimate that blood index of 1-1.5 ppm Hg/g indicates potential risk. Chicks captured on high mercury lakes in Maine had blood index of over 2 ppm Hg/g, and we consider these birds at high risk to Hg exposure. Young loons can depurate their Hg body burdens in three distinct feather molts. Juvenile loons from lakes with lower prey Hg levels (e.g., <0.30 ppm) had a significant accumulation of Hg over time ($p<0.001$), but it was less related to weight ($r^2=0.37$) and also exhibited a slower accumulation rate.



Figure 5. Juvenile Common Loon blood Hg concentrations vs. body weight, Quebec 1997-2001.



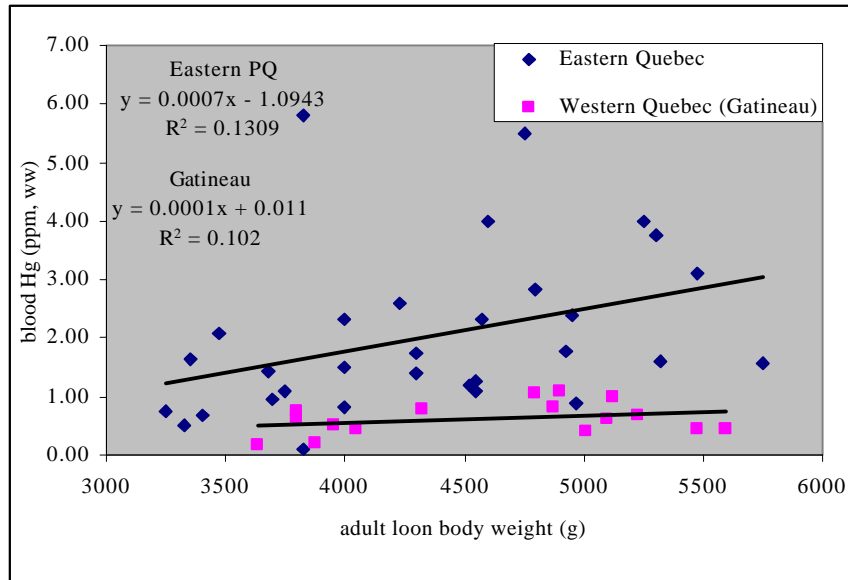
In Quebec, juveniles from Lac du Fou, Lac Main de Fer, and Lac des Iles exceed an index of $2.00 \text{ ppm Hg/g} \times 10^4$ and are at high risk to mercury contamination. La Mauricie and Laurentides area juvenile loons could be at risk from mercury contamination, relative to other Northeastern sites (Table 3). Additional sampling will determine if juveniles in La Mauricie and Laurentides are at risk to the effects from Hg exposure.

Body Size

The mean weights for Quebec male loons were 4611g at LMNP, 4804 in Laurentides, and 5123g at Gatineau and the average female weight was 3707 g at LMNP, 3585 in Laurentides, and 3921g at Gatineau. This is 7% and 8% greater than the mean for Great Lakes' males and females, respectively. In addition, the mean body weights of male and female loons in the eastern regions of Quebec are 7% and 4.5% lighter than the weights of the loons from Gatineau. Generally, there is a west to east pattern of increasing body size in Common Loons. Accordingly, mean mercury concentrations also increase from west to east across North America (Evers et al. 1998, 2003). Loons in New England are approximately 30% larger (using 1992-1996 data) than loons in Minnesota. The mean male and female blood Hg concentrations in New England loons are 2.00 and 1.26 $\mu\text{g/g Hg}$, respectively. In the Eastern Quebec Regions, there is a slight positive relationship between bird weight and blood ($r^2=0.13$) Hg concentrations and in Gatineau where Hg values are consistently low throughout the range of bird weights, there does not appear to be such a relationship (Figure 6). One likely explanation is that Hg exposure in the Gatineau region is too low for such a relationship to emerge.



Figure 6. The relationships between adult loon blood Hg levels and loon body weight, Quebec, 1997-01.



Loon Productivity

Productivity information on breeding loons in LMNP and other regions of Quebec has been collected for past several years. The mean productivity (number of chicks fledged/territorial pair/year) in Eastern Quebec for the past seven years is 0.67 ± 0.35 , $n=23$ territories (range 0.14–1.43) (Table 6). In northern Saskatchewan, stable populations produce 0.535 fledged young/ pair/year (McIntyre and Barr, 1997). The 25-year mean loon productivity for New Hampshire is 0.52 ± 0.09 birds per territorial pair per year (K. Taylor, pers. com.). For comparison, the number of fledged chicks/territorial pair/year in Kejimikujik National Park, Nova Scotia was 0.28, also the lowest productivity among sites currently included in the North American Loon Biomonitoring Program (Kerekes et al. 1995). Many factors may affect chick survival and no apparent relationship was observed between productivity and associated Hg levels for the territories sampled in Quebec.



Table 6. Common Loon Productivity data for the lakes with mercury information, Quebec, 1996-2002.

Number of young produced per year (minimum 3 years of data to make calculations)								(mean)
Lake/Terr.	1996	1997	1998	1999	2000	2001	2002	Moyenne
31 Mile Lake								
Anticagamac	0	1	0	2	1	0	0	0.57
Baie des Onze Iles	0	2	0	0	0	0	1	0.43
Caribou	0	1	1	1	0	0	0	0.43
du Fou ouest	0	2	0	1	0	0	0	0.43
du Fou est	0	1	0	1	0	1	0	0.43
Ecarte	0	0	0	0	1	1	1	0.43
Francais	0	1	0	0	0	0	0	0.14
Helene				2	0		0	0.67
Herman				1			0	
Hughes	1	0	2		1	2	2	1.33
Inman							0	
Lac des Iles (M)		1	2	1	0	0	0	0.67
Lac des Iles (L)		0		1				
Main de Fer					1	2	0	1.00
Pemichangan		2		1				
Reid	1	2	2	1	2	2	0	1.43
Savane-sud				2			1	
Savane-nord							2	
St. Charles nord		1				2	0	1.00
St. Charles sud						2	0	
Souci			1	1			0	0.67
St. Sebastien		2	2	2	0		1	1.40
Thibert					0	1	0	0.33
Tousignant			1	1			0	0.67
Trefle sud	0	0	2	1	0	2	0	0.71
Trefle ouest	0	0	0	2	-	0	0	0.33
Waber	0	1	0	1	1	2	1	0.86
Wapizagonke b1	0	0	2	1	0	1	0	0.57
Wapizagonke b2	0	0	0	0	0	1	2	0.43
Wapizagonke b3	0	0	0	1	2	1	0	0.57
<i>Moyenne (mean)</i>								0.67
<i>Écart-type (std. dev)</i>								0.35
<i>N</i>								23

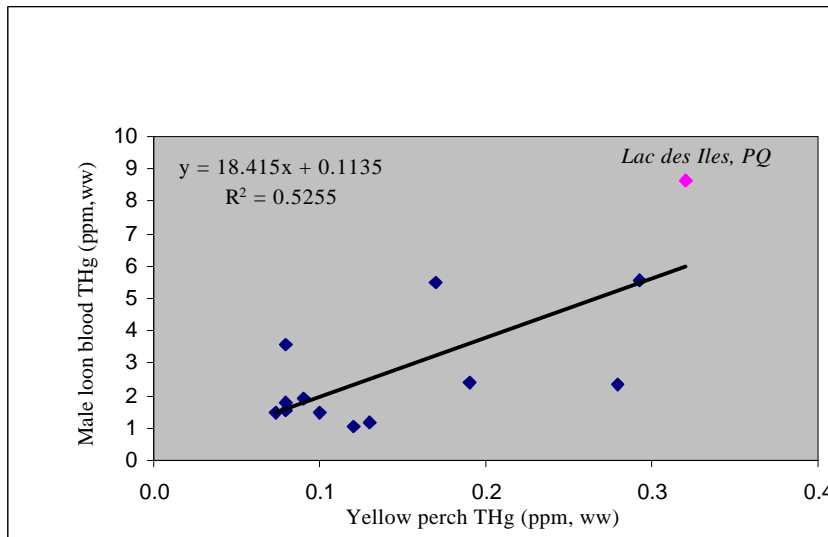


Prey and loon blood mercury concentrations

Lac des Iles case study

Based on our work in Maine we found a positive relationship between yellow perch Hg levels and loon blood. Barr (1986) found that when loons foraged on perch with 0.3 ppm Hg, they had impaired reproduction, and when prey Hg was above 0.3 loons in Ontario had no reproduction. We believe that prey THg concentration that causes impaired reproduction in loons is lower and is around 0.15 ppm, ww (Evers et al. 2002). We included the Hg concentration of large (15-20 cm) Yellow perch from Lac des Iles (0.32 ppm ww) into our regression plot created with Maine data (Evers et al. 2002) to improve the correlation, because loon blood and fish Hg from this lake is higher than the loon blood or fish sampled in Maine (Figure 7). According to the last several years of reproductive success data on Lac des Iles, there were no loon chicks produced in the past three years (2000-2002). It is possible that the combination of water level fluctuations (30 cm), lower pH (6.13), and the air deposition from the industries in Shawinigan, combined contribute to such high Hg levels in the lake's biota.

Figure 7. The relationship between adult male loon blood and Yellow perch (15-20 cm) Hg levels.



Potential effects of Hg on Loon Physiology

Several studies have provided some avian references for no (NOAEL) and lowest observed adverse effects (LOAEL) and LD50 levels. Laboratory or captive experiments with wild birds provide ample information on NOAEL, LOAEL, and LD50 levels (e.g., Fimreite 1971, Heinz 1974, Finley and Stendall 1978, Finley et al. 1979, Heinz 1979, Scheuhammer 1988). While care is essential when applying these effect levels to free-ranging wildlife and for making interspecies comparisons, laboratory experiments provide toxicosis benchmarks and insights for measurable endpoints. Elevated MeHg levels are related to neurological, immunological, and genetic toxicosis (Wolfe and Norman 1998) and disrupt the biochemical functions with cortisol (Friedmann et al. 1996) in fish, cholinesterase in quail (Dieter 1974), and



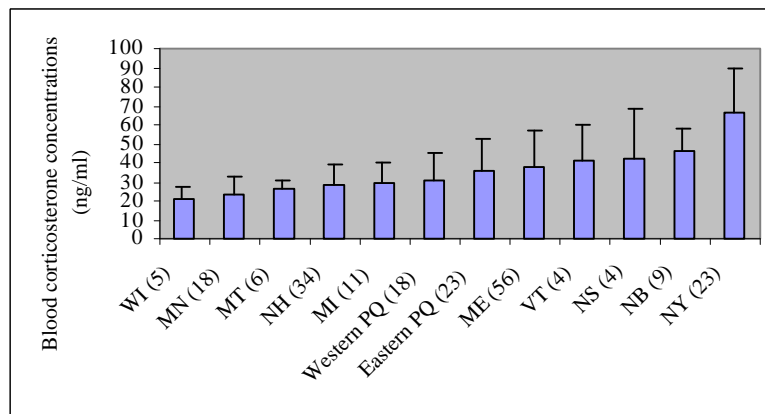
glutathione in mallards (Hoffman and Heinz 1998). In-situ wildlife studies complement many of these laboratory experiments.

In this study, whole blood and blood serum was analyzed to determine potential effects of Hg on the normal functioning of endocrine functions and physiological health. Blood physiological parameters included collected packed cell volume (PCV), blood glucose, plasma total solids, and total white blood cell and differential count. Preliminary analyses of loons sampled across North America indicate mercury levels in adult loons do not correlate with any of the blood physiology parameters measured. However, we did not record elevated WBC counts in any loons with $>5\text{ppm}$ ug/g blood Hg indicating possible immune dysfunction. Additional data are required from loons with high blood Hg concentrations.

Corticosterone, the primary avian glucocorticoid, was measured in Common Loons across the US and from several regions in Canada. The corticosterone release by the adrenal glands is stimulated by the release of adrenocorticotrophic hormone (ACTH) from the pituitary in response to stress. The release of corticosterone can occur within five minutes of ACTH stimulation, and therefore within minutes of stressful stimulus. Corticosterone has been related to capture stress, but can also be related to other factors such as physiology, migration, weather stress, basic health and nutritional status. Recent studies have shown environmental contaminants, such as mercury, cause elevated levels of corticosterone in birds (Dieter 1974) and a decrease in cortisol in fish (Friedmann et al. 1996). Because our processing times and blood collection protocols are similar for all study sites, we examined corticosterone levels in adults.

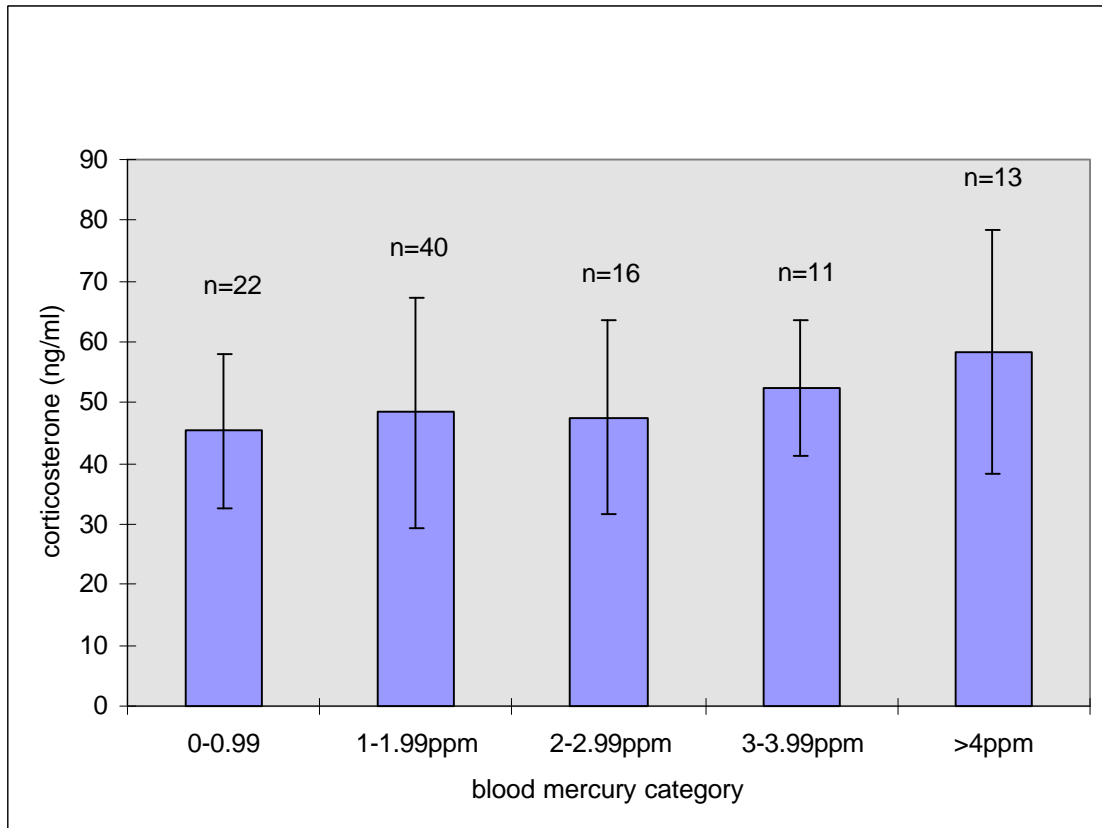
Corticosterone levels analyzed from loons captured in 1997-99 in the Northeast were variable (Figure 8). Eastern Quebec loons had mean corticosterone concentrations of 36.2 ± 16.4 , (range 7.2-60.1 ng/ml).

Figure 8. Mean concentrations of corticosterone in adult Common Loons from selected sites in North America (sample sizes are given in (), bars represent ± 1 SD), 1997-1999.



Mean corticosterone concentrations were slightly higher in birds from high Hg exposure category (Figure 9). Further analyses of corticosterone levels in relation to capture stress, bird weight, incubation period, and processing times need to be completed. To eliminate the possible influence of capture stress on corticosterone levels, in future sampling efforts we will collect blood immediately after capturing loons.

Figure 9. Mean concentrations of corticosterone versus blood Hg concentrations in adult Common Loons from selected sites in North America (bars represent +/- 1 SD)



Risk Evaluation

In this study, we collected a number of tissues from Common Loons that can be used to measure mercury exposure, and to evaluate potential risk to the individuals. One of the few reference studies on piscivorous birds is a study on Hg effects on loons in a lake system highly contaminated from an upstream chlor-alkali plant in Ontario (Barr 1986). Barr (1986) found reproductive impairment in loons (e.g., reduced egg laying and territorial fidelity) feeding on fish with 0.30 ppm Hg and no reproduction in loons feeding on fish with 0.40 ppm or more. Adults exposed to high levels of Hg (i.e., mean brain MeHg levels of 0.76 +/- 0.50 ppm) did not show overt signs of Hg toxicosis. However, mercury levels without



overt signs can still cause a 35-50% decrease in reproductive success (Heinz 1974, Scheuhammer 1987). Evers and Reaman (1997) found that Hg concentrations in Yellow Perch exceed 0.15 ppm (wet weight) in 7 of 8 lakes studied in northern Maine. The authors also documented elevated Hg levels in 18 species of fish preferred by loons (for the three size classes identified by Barr 1996).

Blood and feather are the appropriate matrices to sample if the purpose is assessing Hg exposure in birds. Blood provides an indication of recent dietary Hg uptake. Nearly all Hg in the blood is MeHg bound to erythrocytes and because the half-life of MeHg in avian blood is 2-3 months (Scheuhammer 1988), it is one of the better matrices for determining exposure on a breeding lake. Wolfe and Norman (1998) showed a significant correlation between blood and brain Hg.

Several recent studies have used blood to document Hg exposure in birds. Derr (1995) collected blood from a suite of piscivorous birds in Minnesota, including the Common Merganser. Welch (1994) and Matz (pers. com.) collected blood from juvenile Bald Eagles in Maine. Meyer et al. (1995) and Meyer et al. (1998) in Wisconsin, Scheuhammer et al. (1998) in Ontario, Evers and Reaman (1997) in Maine and New Hampshire, and Evers et al. (1998) across North America have documented elevated exposure levels in loons. Meyer et al. (1998) found that four to eight week old loons with blood Hg levels of 0.30 ppm or higher were associated with territories where fewer chicks hatched or survived to 8 weeks of age. Eleven of the 26 juvenile loons sampled in Eastern regions of Quebec had blood Hg levels over 0.30 ppm. This threshold level is not necessarily useful for other species. Welch (1994) considered 0.50 ppm as a relevant threshold blood Hg level in Maine's juvenile Bald Eagles.

Threshold Hg blood levels in adults are relatively unknown. We have categorized adult loon blood Hg levels based on qualitative observations of effects in the wild and associations with highly contaminated lakes. For example, on Flagstaff Lake in Maine where forage fish mean Hg levels exceed 0.15 ppm, 83% of the adult loon blood Hg levels exceeds 3.0 ppm (n=12). Adult loons with blood Hg levels of 2-3 ppm are considered at moderate risk and those over 3 ppm are at high risk to effects from Hg contamination. Seven adult males out of 22 loons sampled (32%) in Quebec (excluding Gatineau) are in the high-risk category (Figure 10). In the Gatineau area no loons fell into this category.

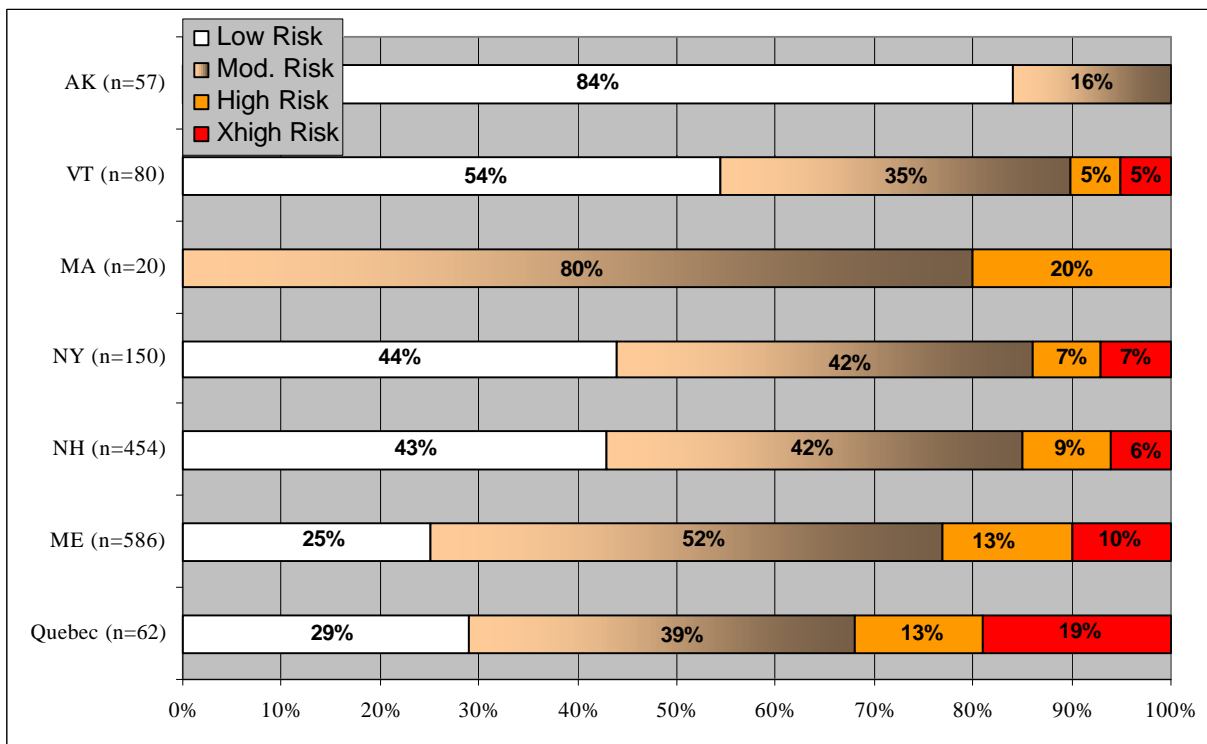
Feathers are indicators of chronic Hg body burden. Evers et al. (1998) found that in recaptured loons feather Hg significantly increased over time, particularly in individuals with elevated blood Hg levels during the breeding season. Mercury ingested with fish during the loon's 6-7 months on the lake is deposited in organs (e.g., liver and kidney) and muscle tissue. Although bile excretion contains some MeHg, most of the MeHg available for remobilization is bound in muscle tissue. This Hg probably reenters the blood stream during stressful times such as full remigial molts. The correlation between flight feathers and breeding season blood Hg levels is strongest in individuals from high Hg regions (e.g. New England $r^2=0.24$), and weakest in birds from low Hg regions (e.g. Alaska $r^2=0.03$).

Feather Hg threshold levels vary according to feather type and bird species. Eisler (1987) considered 5 ppm while Heinz (1979) suggested 9 ppm Hg as a LOAEL. Scheuhammer (1991) and Thompson (1996) consider a higher risk threshold of 20 ppm and the co-authors have observed abnormal behavior in loons with feather Hg above 30 ppm. Four males, or 19%, of the adult male birds sampled exceeded 20 ppm in Quebec (excluding Gatineau). Female loons sampled in Quebec did not exceed the 20 ppm threshold. Adult male loons appear to be most at risk. One juvenile loon captured on Lac des Iles had a feather concentration of 25.7 ppm, the highest concentration we have ever recorded in a hatch year bird.



Mean concentrations in Quebec loons are within the normal range of Northeast samples however certain individuals are at risk to mercury exposure. In particular, close to 50% of the male and juvenile loons exceeded thresholds for given matrices. All loons captured on Lac des Iles are at extra high risk to Hg contamination. These birds had extremely high blood Hg levels but moderate feather concentrations, suggesting that the breeding adults are relatively young birds, and have not had time to bioaccumulate Hg. It would be particularly important to monitor Lac des Iles to see if these birds return to breed. It appears that there is a higher percent of loons at risk to the negative effects of Hg exposure in eastern Quebec than in other regions in North America (Figure 10).

Figure 10. Mercury risk to breeding Common Loons (based on adult and juvenile blood and egg Hg levels)



Genetic Analysis

Genetic analysis of loon samples was conducted by Dr. Amy McMillan at Buffalo State College, Buffalo, New York, and is in the preliminary stage. Seven microsatellite markers were developed during 2001-2002 for use in loon genetic analysis. Microsatellite markers are areas of DNA that contain small (2 to 6 base pairs) tandem repeat units; such as CAA or TA or GC repeated usually 5 to 30 times. Often these segments of DNA are variable in the number of repeat units found in a population. These repeat units are easily discriminated on the basis of their different electrophoretic mobility (McDonald and Potts 1997). These variable segments are extremely useful for population genetic analyses because the mutation rate of these segments is high enough to provide variability within and among populations, amplification with



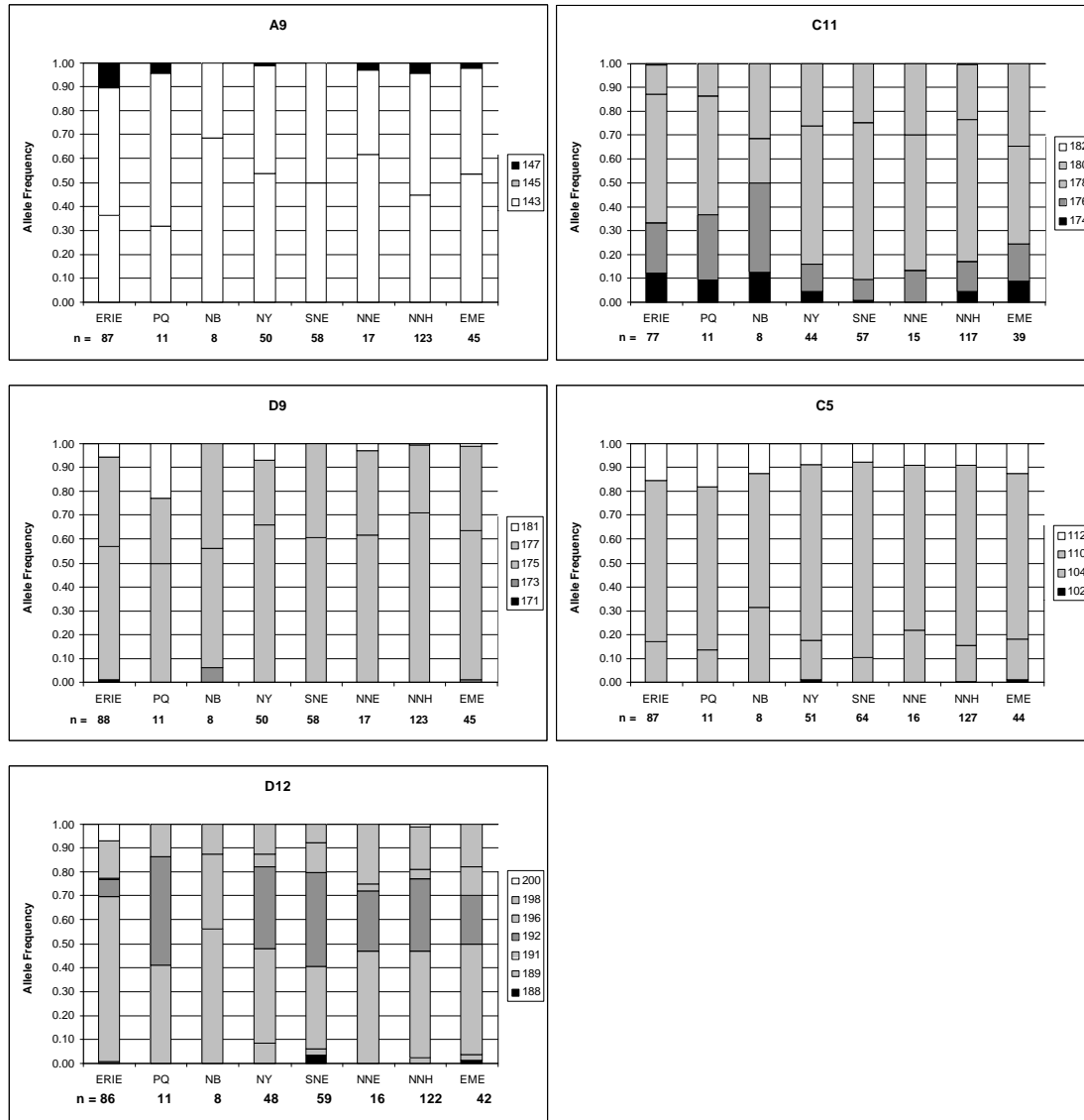
polymerase chain reaction (PCR) is the standard procedure, which allow for small sample volumes, and they are codominant (both alleles are visible), neutral markers.

Eleven birds of the 1999 and 2001 samples from Quebec have been processed; one bird each from Thibert, Hughes, and Lac du Fou (2001) and one from Pemichangan and seven from 31 Mile (1999) (Figure 11). Preliminary analysis indicates allele frequency variation across the eastern regions of Canada and the United States (Figure 11). These results are extremely preliminary and, except for an analysis of allele frequencies across regions, no other analysis has been performed. BRI has three samples from 1998, 23 more from 1999 and 11 more from 2001 available for analysis that were not included in the initial microsatellite screening. Further analysis will be done in the coming year after the inclusion of the rest of the Quebec samples and the addition of samples from other regions. In 2003, we plan to identify and analyze additional microsatellite loci for common loons and to explore the use of a second type of genetic marker, AFLP (Amplified Fragment Length Polymorphism), for use in quantifying the genetic structure of loons.

Our long-term goals with the genetic analysis are to 1) quantify the genetic structure of loons across the summer breeding territory in North America, 2) link these breeding populations with wintering loons along the coastal zones of North America. Accomplishing these goals will allow us to determine the impacts of both summer and winter stresses on loon populations. We hope to be able to determine where loons originated when birds are recovered from coastal oil spills in the winter months and to analyze birds killed by botulism poisoning in the Great Lakes for breeding populations of origin.



Figure 11. Preliminary allele frequency distributions of five representative microsatellite loci designated A9, C11, D9, C6, and D12. Erie = migratory birds - Lake Erie (botulism deaths collected in New York, 2001); PQ = Quebec - 1999 and 2001; NB = New Brunswick -1996; NY = New York - 1999-2001; SNE = southern New England – Massachusetts and southern New Hampshire – 1997-2001; NNE = northern New England – northern Vermont and north-western New Hampshire – 1998-2001; NNH = north-eastern New Hampshire and northwestern Maine – 1997-2001; SME = south and east Maine – 1997-2001. Sample size (n) = number of birds represented. Each bird contributes 2 alleles to the allele frequency calculations.



Recovery information

Three of the loons banded in the Eastern Region of Quebec were recovered dead on the coast of North Carolina in late winter-early spring (Table 7). The cause of death was not determined. These recoveries suggest that Quebec's eastern population of Common Loons overwinters in North Carolina. One loon banded in the western region, on 31-mile lake (Long Island Sound Territory), was recovered in Florida.

Table 7. Recovery locations of common loons banded in Quebec in 1997-2001.

Lake banded	Date banded	Age	Sex	Recovery Location	Recovery state/province	Recovery Date
Lac du Fou	7/25/97	Juvenile	Unknown	Outer Banks	North Carolina	Feb-1998
Tousignant	8/13/98	Adult	Male	Tousignant	Quebec	8/28/98
31-Mile Lake	8/1/99	Adult	Male	Ormond Beach	Florida	12/31/01
Wapizagonki	8/11/99	Adult	Male	Topsail Isl. Beach	North Carolina	4/7/01
Hughes	7/30/01	Juvenile	Unknown	Ocracoke Island	North Carolina	4/5/02

Future Recommendations

Future research should emphasize further color-marking and sampling adult and juvenile loons in the eastern regions of Quebec. Blood and feather Hg levels from these individuals will increase the usefulness of known contaminant values and improve regional comparisons. Previously marked adults should be monitored for productivity and survivorship (especially the LMNP males with elevated feather Hg levels, and loons on Lac des Iles) and ideally, followed through a one-year cycle using satellite transmitters. Additional research should focus on the relationship between Hg concentrations in piscivorous birds, their prey, and lake water chemistry and morphometric features. If possible, annual productivity information for each territory and each banded individual should include the number of nesting attempts and the number of downy young produced in addition to the number of chicks that fledge successfully.

Acknowledgements

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