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Assessing the impacts of methylmercury on piscivorous wildlife as indicated by the Common Loon



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Abstract

Anthropogenic inputs of mercury (Hg) into the environment have significantly increased in the past few decades. In conjunction, the current availability of methylmercury in aquatic systems has increased to levels posing risks to human health and wildlife populations. Risk levels vary considerably in response to methylmercury availability, which is affected by lake hydrology, biochemistry, and may be associated with topography and proximity to airborne point sources. We selected the Common Loon as the most suitable bioindicator of aquatic Hg toxicity, based on ecological, logistical, and other criteria, including public valuations of natural resources.

Non-random sampling efforts from 1994-98 indicate New England's breeding loon population is at unacceptable levels of risk to Hg contamination, particularly in Maine. Based on risk categories developed from the literature and *in situ* studies by BRI and their collaborators, 21-35% of the breeding loon population in Maine is estimated to be at risk, while 80% of the eggs laid exceed safe Hg levels and 38% are likely impacted. Because the evidence indicating loons were at serious risk from Hg was substantial, we identified several new organism and population parameters in order to increase confidence in our defined risk thresholds.

Between 1994-98, we collected 78 abandoned eggs and blood and feather samples from 140 adult and 54 juvenile wild loons monitored and captured in Maine. In 1998, 60% of the total samples were collected and analyzed. The Hg concentrations of these samples were used to analyze potential subtle impacts of Hg on a number of parameters including effects on reproductive success, egg viability, behavior, developmental stability, immunosuppression, and individual survival. A total of 92 loon territories were monitored on 13 lakes in 1998, and behavioral studies were conducted on 33 loon territories.

Overall productivity had a tendency to decline as adult blood Hg levels increased, thereby corroborating other studies that show Hg impacts. We did not find significant differences among five established egg development stages, however the majority of eggs collected had potentially toxic Hg levels and Maine's mean egg Hg concentration (1.00 +/- 0.55 ppm, ww) was higher than means from seven other states. The impact of high loon prey Hg levels on adult behaviors, tended to be evidenced through greater time spent on nest and more time brooding instead of actively foraging for their young. Data from the time-activity budgets quantifying chick behavior indicate adult loons spend less time swimming in high Hg risk territories than low ones.

We estimated chronic Hg impacts on individual survival through the recapture of adults and found Maine loons have annual Hg increases of 10.6% and 7.5% for males and females respectively. We predict life expectancy and reproductive individual performance to be potentially abbreviated for adults breeding in Maine and other high risk areas. Further, young-of-the-year loons have increasing blood Hg levels from at least 3-12 weeks of age on high risk territories, but not on lower risk territories.

Several biomarkers of Hg exposure were also developed. Although blood profiles (which quantify blood characteristics, such as white blood cells) did not show a relationship with blood Hg levels, we did find compelling evidence that loons are being affected by elevated environmental Hg. Developmental instability, measured through feather asymmetry, of adult loons from breeding populations at high risk to Hg levels in New Hampshire and Maine was significantly greater than lower risk breeding populations in Vermont, New York, and the Great Lakes. While corticosterone levels, which reflect various environmental and physiological stressors, were significantly higher for loons with blood Hg levels over 4 ppm.

Further studies building from this work will provide more definitive risk assessments that are based on Hg impact thresholds developed in Maine, and will help confirm if the effects we documented are persistent. If the risk to Maine's breeding population is substantial, investigations into other piscivorous wildlife may be warranted, providing a more complete picture of Hg's effects on the Maine environment and human health.

Introduction

Due to high concentrations of mercury (Hg) in fish from Maine lakes, ponds, rivers, and streams, the Maine Bureau of Health issued a statewide "fish consumption advisory" in 1994, (modified in 1997) warning Maine citizens to limit consumption of fish from all fresh waters. Impacts on wildlife, however, are less well known in Maine and elsewhere. Recent ecological concerns were highlighted at the "New England Governors and Eastern Canadian Premiers" conference sponsored by the U.S. EPA and Maine Department of Environmental Protection. Recommendations from the report 'Northeast States and Eastern Canadian Provinces Mercury Study: A Framework for Action' stated: "conduct additional research on the cycling and bioavailability of mercury in aquatic ecosystems and on the ecological impacts of elevated fish mercury levels, particularly for fish-eating wildlife such as eagles, loons, osprey, otter, and mink" (NESCAUM 1998).

In addition, strategy 9 from "Mercury in Maine," a report by the Land and Water Resources Council to the Maine legislature in January 1998, recommends "focus biological research efforts on the effects of mercury on the health of loons, fish and other wildlife with elevated mercury levels." Emphasis from policy makers and researchers has been on higher trophic level piscivorous wildlife since they are most at risk due to mercury's ability to bioaccumulate and biomagnify (Scheuhammer 1991, Thompson 1996, U.S. EPA 1997).

Using Birds as bioindicators of MeHg availability

The use of piscivorous birds as indicators of MeHg availability is common (e.g., Fox 1994). We believe piscivorous birds are also useful as general ecological indicators of aquatic ecosystem integrity and of the presence and effects of environmental stressors.

Mercury deposition and MeHg availability is now sufficiently elevated in the Northeast region to cause impacts on wildlife (Welch 1994, Burgess et al. 1998, Nocera and Taylor 1998). Based on U.S. EPA probability-based sampling efforts in U.S. EPA's Region 1 and 2, Yeardley et al. (1998) predicted that 98% of New England's lakes contained fish with MeHg levels exceeding critical values for piscivorous birds. In corroboration, Evers et al. (1998a) found Common Loons (*Gavia immer*) breeding in Region 1 (MA, ME, NH, RI, VT) had the highest mean blood Hg levels in the United States, while juvenile loon blood Hg levels were four times those at the designated reference site in Alaska. Further studies on a suite of five piscivorous birds in Maine indicated over 70% of lakes have the capacity to produce MeHg at levels above designated risk categories (Evers et al. 1998b). These studies demonstrate that extensive Hg contamination and MeHg availability exists in Region 1.

Yeardley et al. (1998) found from analyzing 11 metals in fish throughout the U.S. that, "MeHg was determined to be the elemental contaminant of regional concern to fish consumers." This study focused on assessing the ecological risk of Hg in a piscivorous bird—the Common Loon. We chose the loon as our bioindicator because there exists a significant amount of information collected on its demographics (e.g., Evers et al. 1999, Piper et al. 1997a, Piper et al. 1997b), behavioral ecology (e.g., Evers 1993, Nocera and Taylor 1998, Paruk 1999), toxicology (e.g., Evers et al. 1998a, b, Meyer et al. 1998, Scheuhammer et al. 1998), and local breeding population status (Maine Audubon Society and BioDiversity Research Institute unpubl. data).

Hg risk in loons

An estimated 21-35% of the New England Common Loon breeding population has Hg levels that exceed wildlife safety thresholds designated by other studies (e.g., Barr 1986, Scheuhammer 1991, Thompson 1996, Burgess et al. 1998, Meyer et al. 1998). In addition, over 60% of abandoned loon

eggs collected in Region 1 (n=305) have Hg levels considered elevated (i.e., 0.5 ppm) by laboratory studies (Fimreite 1971, Heinz 1979) and 5% have lethal levels (i.e., 2.0 ppm) (Thompson 1996).

These and previous studies documenting exposure in Maine loons (Evers and Reaman 1998, Evers et al. 1998b) predict that impacts are likely. This study was initiated in order to (1) determine the extent of actual impacts on loons in Maine's lakes and ponds and (2) tighten impact thresholds used to establish risk. We and other collaborators believe relationships exist between high Hg levels and: (1) decreased egg laying capability, (2) decreased egg hatchability, (3) altered parental investment, (4) altered chick behavior, (5) reduced fitness in adults and juveniles, (6) decreased juvenile survival, and (7) reduced lifetime reproductive success. Complementary collaborative studies in the Great Lakes, other New England states, and Canadian Provinces aid in interpretation of our results.

Study Areas

BioDiversity Research Institute (BRI) has collected exposure, demographic, and physiological information on New England's breeding loon populations since 1994. Much of this research has been based in the upper Androscoggin and Kennebec River watersheds (e.g., Evers and Reaman 1998, Evers et al. 1998b). Because of this knowledge base and some of the highest Hg levels recorded for Common Loons in North America, we chose the Rangeley Lakes area for a high resolution study on the potential impacts of Hg to wildlife (Figure 1). All lakes are in Maine except for parts of Lake Umbagog and Round Lake in New Hampshire.

The type of lake was classified as natural or impounded by dams. We monitored 92 loon territories on 7 impoundments and 10 loon territories on natural lakes (Table 1). Impoundments were defined according to their water management regime by Evers and Reaman (1998): Regulated storage reservoir (RSR) had annual fluctuations greater than 1.5 m, regulated peak reservoirs (RPR) had weekly water fluctuations over 1 m, while regulated full ponds (RFP) were raised lakes managed for minimal water level fluctuations of less than 1.5 m. Drainage lakes had both an inlet and outlet where the primary water source is stream drainage while spring lakes have no inlet but do have an outlet. The water source for spring lakes is the groundwater flow from the immediate drainage area.



Table 1. Common Loon territories monitored for overall reproductive success and behavior.

Lake	Lake Type[1]	# monitored terr. overall success (A)	# monitored for terr. behavior (B)	Hg risk category (# monitored terr. for A and B)			
				Low	Mod	High	XHigh
Aziscohos	reservoir-RSR	17	8	0, 0	5, 3	3, 3	9, 2
Chain-of-Ponds	drainage	3	2	0, 0	1, 1	1, 0	1, 1
Flagstaff	reservoir-RSR	21	6	0, 0	4, 0	6, 0	11, 6
Jim	drainage	3	3	1, 1	2, 2	0, 0	0, 0
Little Beaver	spring	1	1	0, 0	1, 1	0, 0	0, 0
Little Jim	spring	1	1	1, 1	0, 0	0, 0	0, 0
Mooselook	reservoir-RSR	2	0	1, 0	1, 0	0, 0	0, 0
Quimby	spring	1	1	1, 1	0, 0	0, 0	0, 0
Rangeley	reservoir-RFP	9	3	7, 2	2, 1	0, 0	0, 0
Richardson	reservoir-RSR	4	1	2, 1	2, 0	0, 0	0, 0
Round	spring	1	1	0, 0	1, 1	0, 0	0, 0
Umbagog	reservoir-RSR	24	6	5, 1	15, 1	4, 4	0, 0
Wyman	reservoir-RPR	5	0	0, 0	0, 0	4, 0	1, 0
TOTAL	13 lakes	92	33	18, 5	34, 9	18, 7	22, 9

[1] RSR (regulated storage reservoir), RFP (regulated full pond), RPR (regulated peak reservoir) are impoundments while spring and drainage lakes are natural.

Methods

Samples collected from lakes in other New England states, the Great Lakes region, and the Canadian Maritimes, were used in regional comparisons and for measuring some Hg effects endpoints. We categorized loon territories on single and multi-territorial lakes according to known exposure to MeHg (indicated by blood or eggs). The four risk categories were based on literature and *in situ* studies by the authors and their collaborators for three matrices (Table 2). Low risk indicates background Hg levels that are minimally impacted by anthropogenic inputs. Loon territories that are in the moderate risk category have elevated MeHg availability but levels most likely do not impact individuals. Individual loons that are designated in the high risk category are exposed to toxic levels of environmental Hg that potentially have molecular, organism, and/or population effects. The extra high Hg category is designated based on known impacts on loons and other birds.

Table 2. Risk categories for MeHg (ppm) availability in the Common Loon

Matrix	Low	Moderate	High	X High	Reference Base
Egg	0-0.5	0.5-1.0	1.0-2.0	>2.0	Barr 1986
Blood-Adult	0-1.0	1.0-3.0	3.0-4.0	>4.0	BRI[1], inferred by Barr 1986 [2]
Blood-Juv.	0-0.1	0.1-0.3	0.3-0.4	>0.4	Meyer et al. 1998[3]
Feather	0-9	9-20	20-35	>35	Thompson 1998, BRI[1]

[1] BRI refers to unpublished data by BioDiversity Research Institute
[2] Adult blood Hg levels are generally 10x higher than prey Hg levels (Evers and Reaman 1998) and Barr 1986 found lower reproduction of loons with prey Hg levels of 0.3 ppm and no reproduction at 0.4 ppm.
[3] Applies to 3-5 week-old juveniles, only.

Although we monitored 92 territorial pairs in 1998, the sampling basis for the following six parameters varied geographically and temporally. For objectives 1, 3, and 4, all information originated from the territorial pairs in Table 1. Objective one also included information from 26 New Hampshire territories. Our analysis for objective 2 was based on eggs collected in Maine from 1994-98. Data for objectives 5 and 6 were from adult and juvenile loons captured from 1994-98.

1. Impacts on Overall Productivity

We surveyed nesting and non-nesting territorial loon pairs on 92 lake territories from ice off (early May) until mid-September (Table 1). Surveys consisted of locating loon pairs every 3-5 days from a boat with 10x binoculars, documenting territorial duration, nest bowl attempts, number of eggs laid, incubation efforts, and causes for nest abandonment/failure. Human disturbance, evidence of predators, and frequency of intruding loons were also documented at this time. Reproductive information from 1998 was compared with historical information from our study lakes (e.g., Fair 1997) and an 8-year New Hampshire productivity database from the Loon Preservation Committee (Taylor pers. com.).

2. Impacts on Egg Development

Whenever possible, biologists would collect whole eggs from nests that had been abandoned, predated or flooded. Eggs were only removed from a site when the adults were no longer incubating them, or they were determined inviable (i.e., strong odor, or indications that eggs were not turned). Eggs were placed in a polyethylene bag and labeled with lake and territory name, date, and collector while in the field. Eggs were then frozen as soon as possible. Later, eggs were measured for length, weight, and volume. Volume was measured by water displacement and weighed on an electronic balance to the nearest 0.001g. Egg weight was also weighed to the nearest 0.001g. The egg length and width were measured with calipers to the nearest 0.001 mm. Eggs were cut open with a scalpel and the contents were placed into sterile I-Chem® jars (including as much of the egg membrane as possible). The contents were then categorized into one of the developmental stages (Table 3). Egg contents were homogenized and shipped to the University of Pennsylvania laboratory (supervised by Dr. Robert Poppenga) and analyzed for total Hg (wet weight) using CVAA (see Evers 1998b for methods).

Table 3. Embryological developmental scale used for Common Loon eggs.

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, whereas a yellow homogeneous liquid may be sifted through and if no dark spots or hardened areas were found we classified the egg as infertile (0).
0:	No development was 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 may be visible at this stage.
2:	Developing embryo (length was 1.5 – 2.0) has an apparent central nervous system. Cranial development and visible eyes are apparent. Feathers are absent.
3:	The embryo shows advanced development (length was 2-3 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. Appendages were completely developed. Vent, preen gland was visible. A small portion of yolk sac remained attached to belly.

3 & 4. Behavioral Impact Assessment for Adult and Juvenile Common Loons

We collected a total of 700 hours of adult and chick loon behavior observations from 33 territories on 13 lakes in Maine (and Round Lake, NH) between early May and late August for the loon's pre-nesting, nesting, and post-nesting periods. Loon territories were placed into extra high, high, moderate, and low risk categories (Table 1). We collected behavior data using time-activity budget (TAB) methods based on those described by Altmann (1974), Tacha et al. (1985), and Nocera and Taylor (1998). Observation periods were not staggered throughout the photoperiod due to findings by Evers (1994), Gostomski and Evers (1998), Mager (1999), and Paruk (1999). They found minimal or no significant relationships between time of day and behaviors.

Individual loons were observed in one hour time blocks for up to 4 hours/day using one or two 15-45X spotting scopes and 10X binoculars. Observers (who would continually monitor behavior through a spotting scope) relayed behaviors to a recorder, who noted times from a digital stopwatch and recorded categorized observations on data sheets. Observers and recorders also monitored for intruding and non-intruding loons, boats, and predators. Data were collected by a combination of six BRI biologists and trained EarthWatch Institute Volunteers. Martin and Bateson (1993) addressed potential problems with observer bias and misinterpretation of behaviors. Observer bias was minimized in this study by training all BRI biologists simultaneously for 3-4 days, and meeting several times throughout the season. EarthWatch Institute volunteers, although trained, were generally designated as recorders and observed only with the supervision of BRI biologists. Bradley (1985) addressed the importance of minimizing visibility and discovery bias when collecting TABs. We addressed these potential biases by concealing ourselves and/or through remote observation (up to 300 m).

Explanation of Time-Activity Budget Methods

The time-activity budget method is a continuous sampling method (Tacha et al. 1985) which better represents and quantifies behavior data than either instantaneous and/or nonfocal animal sampling methods (Martin and Bateson 1993). Behavior data was classified into two different categories: behavior states and behavior events (Altmann 1974, Tacha et al. 1985, and Nocera and Taylor 1998).

Behavior states were timed behaviors and defined by lasting more than 20 seconds. Examples of commonly seen adult behavior states were foraging (for self or chicks), locomotion, preening, sleeping, nest sitting, drifting and brooding. Commonly seen chick behavior states included swimming, sleeping, back riding, under wing and preening (Appendix I).

Behavior events were counted behaviors defined as lasting less than 20 seconds. Events that lasted more than 20 seconds were then timed as behavior states. Examples of commonly seen adult behavior events were wing flaps, egg turning, chick feeding, peering, and foot waggles. Commonly seen chick behavior events include foot waggles, head shakes, peers and wing flaps (Appendix I).

Differences exist in previous studies of how to categorize and analyze periods where the subject was out of sight. Evers (1994) and Gostomski and Evers (1998) collected TABs within one hours continuous time blocks. During a TAB, loon behaviors that could not be viewed were classified as "out-of-sight". They assumed loon behaviors were not significantly different than in-sight behaviors which avoided potential observer bias. Nocera and Taylor (1998) chose to remove it and reduced the sampling period accordingly. In this study, we collected behavior data continuously until a total of 60 minutes was recorded per individual except in cases where the subject was out of sight for more than 20 minutes. Observation periods that were less than 40 min per individual were not used in the final analysis. Therefore, although the "out-of-sight" behavior category was not incorporated, 60 minutes of continuous observation was generally not made.



In cases where observers felt they could not adequately record behaviors of all target loons present, selected individuals were excluded from observations according to priority. Chick behaviors held the highest priority, while adults were second, based on chick behavioral effects documented by Nocera and Taylor (1998).

Distinguishing/identifying individuals

In order to compare behaviors among individuals in different mercury risk categories (extra-high, high, moderate, and low), absolute identity of the individual being observed was crucial. The potential for confusion of individuals within a territorial pair was much higher than between different pairs due to exclusive territorial behavior and high site fidelity (Evers et al. 1999).

Adults: In almost all cases, at least one or both individuals of the territorial pair were marked (color banded and bill and/or feather marked), which allowed consistent positive identification of the loon being observed. Bill paint and feather markings were often visible from a great distance. Colored leg bands were easily observed above water during nest sitting and comfort/maintenance behaviors, and were often visible underwater while swimming and foraging with binoculars or a spotting scope. Individuals that were not positively identified were not used in the final analysis.

Chicks: Most of the observed territorial pairs hatched only one chick or only one chick survived the first few weeks. These cases tended to have less logistical complications associated with positive and consistent identification of individuals throughout the duration of the TAB than two-chick families. Individuals from the same clutch will typically stay within close proximity to each other and are essentially identical from our observation range. Observers performed TABs on either chick or both, depending on the number of available observers and recorders. We addressed the possibility of confusing the two chicks during the observation period in one of two ways. Whenever possible, one chick was captured and either banded (if > 3 ½ weeks), and/or bill marked (with a orange paint that would degrade in several weeks) so that both individuals could be easily distinguished from a distance. When chicks were not marked, we chose one chick randomly whenever we lost track of an individual, and then continued observations.

Nest Sitting: Monitoring with Data Loggers

Temperature data loggers were used to discern adult behavioral effects as related to nesting behavior. Deployment sites were chosen according to logistics and designated mercury risks categories—a range of low and high sites were chosen. Two types of BoxCar® Pro (Onset Computer Corporation, Bourne, MA) data loggers were used. The Stowaway® XTI logger has a single temperature sensor at the end of a flexible wire, while the Hobo® H8 Pro Series Logger has a dual monitoring system. This system involves one sensor on the base of the unit to monitor ambient temperature, and one sensor at the end of a flexible wire to monitor nest temperature.

The main unit of the data loggers was placed at a site near the nest that received a similar amount of direct sunlight as the nest. Temperature data loggers were inserted into nests that already contained eggs. The flexible probes were inserted into the nest by coming up through the bottom of the nest material and placed 1-2 cm below the egg within the nest material. Post-installment nest checks confirmed the temperature sensors were held firmly in place by the covering material. The data loggers were programmed to take temperature measurements every 40 seconds, and were deployed for 1 to 19 days. Information from the data loggers was interpreted using overlapping TABs.



5. Impacts on Individual Survival

We attempted to determine impacts on adult and juvenile individual survival in two ways: 1) regular visits to territories until ice-on and 2) recapturing previously marked adults.
Due to its opportunistic nature, regular visits to territories throughout the season gives limited but useful insights on individuals' survival. Through consecutive visits to a territory, were able to evaluate risks associated with that location (e.g. predators, intruders, high wave action) and continually check on the status of the territorial pair and young.

Recapturing previously banded and sampled loons allowed us to look at accumulation rates of mercury within individuals over time. Whereas blood mercury reflects recent dietary uptake, feather mercury levels are more indicative of chronic accumulation rates (Burger 1993).

The chick age class distinction is designed to follow the developing loon's molt pattern, which affects the amount of mercury available in the bloodstream. Young-of-the-year loons feed on lakes for their fist 14-20 weeks and 4-10 weeks of that time is after their last feather molt (depending on ice-on). Mercury is depurated into these newly molted gray feathers, thereby lessening the overall body burden of mercury (Burger 1993). Therefore, we captured young loons at different periods of their molt from similar Hg risk areas of Aziscohos and Flagstaff lakes.

6. Physiological Impacts

Six blood parameters were measured in adult and juvenile blood from 1995-98 at sites throughout North America: packed cell volume, glucose, total solids, white blood cell counts, and white blood cell types (differentials). Standard methods were used for each measurement. Corticosterone and estrogen levels were measured with standard assays by Tufts University.

For determining feather asymmetry we cut the second secondary at a standard site on the rachis where the calamus meets the feather's vane to standardize measurements of length and weight. Precision of measurements were 0.001 grams (weight) and 0.01 mm (length).

Results and Discussions

We believe investigating multiple levels of biological organization (e.g., genetic, organismal, and population) for the Common Loon provides the quantitative benchmarks needed to evaluate environmental stressors like Hg. The following sections describe our techniques for six targeted parameters.

1. Impacts on Overall Productivity

Barr (1986) found a strong negative correlation between the successful use of a territory by breeding pairs and Hg contamination (measured in forage fish). Loon reproduction, capacity to lay eggs and maintain nest/territory fidelity, was impacted with fish Hg levels of 0.3 ppm and no reproduction occurred in areas with fish Hg levels of 0.4 ppm. Burgess et al. (1998) also found a significant negative correlation between loon blood Hg levels and impacted reproductive success by using the ratio of nesting vs. territorial pairs (i.e., detects whether an egg was laid).

We found no correlation between the four reproductive success ratios and adult blood, juvenile blood, and egg Hg concentrations ($p>0.05$). However, with higher mercury risk categories there was a significant decrease ($p<0.05$) for each reproductive index ratio, except the nesting/territorial pair ratio (Table 4).

We determined the reproductive status of 92 Maine territories for three parameters: egg laying, egg hatching, and chick survival. These reproductive parameters were then related to blood Hg levels associated with the representative breeding territory.

Table 4. Reproductive success of Common Loons in the Rangeley Lakes Region, Maine and parts of New Hampshire

Level of risk by territory	Mean Risk	Mod Risk	High Risk	x-high Risk
Nesting/Territorial Pair Ratio	0.76	0.76	0.77	0.76
Hatching/Nesting Ratio	0.86	1.15	0.61	0.45
Fledging/Territorial Pair Ratio	0.77	0.89	0.70	0.50
Fledging/Nesting Pair Ratio	0.57	0.89	0.52	0.37
Sample size (territories)	63	32	20	11

Further sampling is required because reproductive success is impacted by several factors not accounted for by the 1998 data collection. One significant variable is the influence of water fluctuations on loon's nesting on reservoirs and flashy natural lakes. Productivity is also heavily determined by individual fitness (Newton 1992), loon mate replacement rates of approximately 20% annually (Evers et al. 1999), and the nonbreeding New England population of 46% (Taylor and Vogel 1999). Therefore, there is a need to monitor individual performance of color-marked loons to analyze the impacts of these confounding factors.

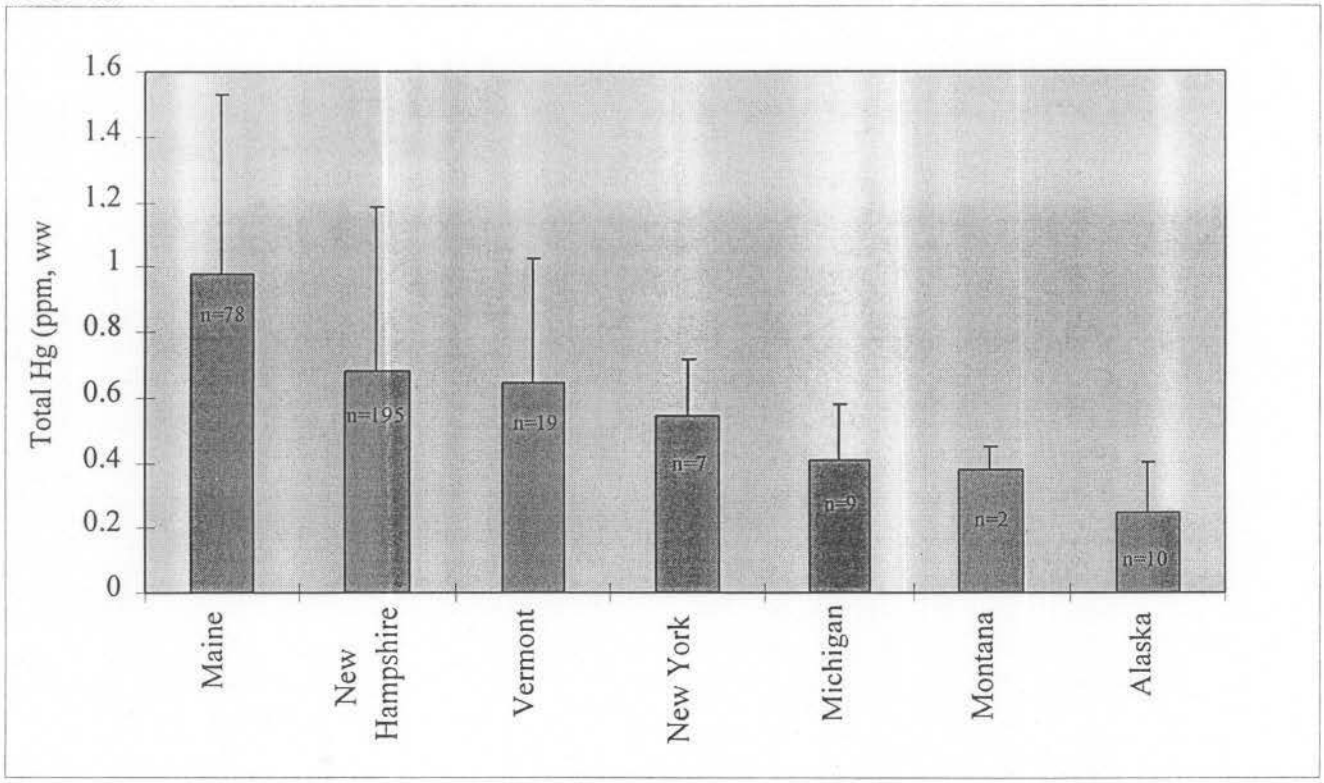
2. Impacts on Egg Development

Controlled studies have shown that mercury toxicity impacts egg development and hatchability at levels (i.e., 0.5-2.0 ppm) that are found in this study (Borg 1969, Fimreite 1971, Spann et al. 1972, Gilbertson 1974). Lower reproductive success in birds has been documented at Hg levels lower than those that cause observable effects on adult behavior and survival (Scheuhammer 1991). Thompson (1996) summarized several controlled studies of captive birds and predicted dietary concentrations of 0.6 ppm (wet weight) (converted from 3.0 ppm dry weight) causes impaired reproduction in birds yet has little effect on adults. Barr (1986) found loons laid fewer eggs when prey averaged 0.3-0.4 parts per million (ppm) and no eggs were laid when prey averaged over 0.4 ppm.

We collected eggs and associated morphometric and developmental information from 78 eggs that were abandoned on Maine lakes because of flooding, human disturbance, and unknown reasons. Loons typically lay two eggs within 24 hours of one another. Mean egg size in Maine was 145.9 +/- 22.1 g (weight), 91.9 +/-3.8 mm (length), 56.9 +/- 2.4 mm (width), and 140.6 +/- 19.8 g (volume). In birds, first-laid eggs are larger than ones following. We ran a discriminate function analysis (DFA) on 21 egg clutches from Maine and New Hampshire loons for the four morphometric measurements and found length to be the best predictor variable (72% correct) of egg laying order. If DFA can predict the largest egg (in nests where one egg hatches and one does not) we can then classify egg-laying order in one-egg collections as well as two-egg ones. This would help reduce predictive errors with one egg clutches since we have found mean Hg concentrations between eggs in a clutch to vary by 36%.

Maine loons had egg Hg concentrations with a mean of 1.00 +/- 0.55 and ranged from 0.118 to 2.65 ppm (wet weight). Except for New Hampshire, mean egg Hg concentrations were significantly higher ($p<0.05$) than other North American study sites in Alaska, Montana, Minnesota, Michigan, New York, and Vermont (Figure 2). New Hampshire mean egg Hg concentrations tended to be lower than Maine's.

Figure 2. Mean Hg concentrations in Common Loon eggs from selected sites in North American, 1993-98.



We did not find egg Hg to significantly differ between fertile and nonfertile eggs ($p>0.05$). Spann et al. (1972) found lower survival during the third trimester of pheasant embryos, loon egg Hg concentrations did not significantly differ between five developmental stage categories ($p>0.05$). However, since over 80% of Maine's loon eggs have Hg concentrations at levels that other studies have shown impact, we plan to develop a DFA that can better predict egg laying order for nests that have two-egg clutches and reduce confounding variables.

3. Adult Behavioral Impacts

Precedence for measurable Common Loon behavior abnormalities has been documented during incubation (BioDiversity Research Institute, unpubl. data) and evidence indicates reproductive impairment is associated with high mercury exposure (Burgess, 1998), suggesting elevated Hg concentrations can have an effect on adult behavior and ultimately reproductive success. In this study, we quantified adult behavior through time activity budgets (TABs) during three distinct breeding periods: pre-nesting, nesting and post-nesting. These data were not directly comparable with time-activity budgets gathered in the Midwest in Michigan's Seney National Wildlife Refuge (Evers 1994),

Isle Royale National Park (Gostomski and Evers 1998), western Upper Peninsula (Mager 1997), and in Wisconsin's Turtle Flambeau-Flowage (Paruk 1997) because we preferentially selected incubating adults and those attending young. However, we can compare gender differences during the breeding periods and relate results to these studies.

Gender differences in behavior

During the pre-nesting period we found adult males spent more time preening than females, while females foraged more (Table 5). Loon behavior studies in the Midwest indicated no significant difference in preening or foraging during this period. Adult male loons have significantly higher levels of mercury than females (Evers et. al, 1998 (SETAC) and 26-31% males in Maine are at risk from mercury exposure compared to 12-21% in females. (BRI, unpubl. data), based upon feather and blood Hg concentrations. Additional data is necessary to determine the relationship of these findings with mercury concentrations.

Table 5. Percent of time spent in seven behavioral categories during three breeding periods in Maine, 1998

	Pre-nest (n=49hrs)		Nesting (n=104hrs)		Post-nest (n=148hrs)	
Activity	Male	Female	Male	Female	Male	Female
Foraging	42	58	15	11	7	5
Resting	10	12	4	1	3	<1
Locomotion	14	11	9	6	3	4
Preening	17	3	6	4	2	2
Courtship	<1	1	-	-	-	-
Nest Sitting	-	-	62	76	2	8
Chick rearing	-	-	-	-	79	74
Other	16	15	4	2	4	6

During the nesting period, adult females spent a greater proportion of time incubating and less time feeding than males (Table 5). In the Midwest, the percentage of time spent incubating by each sex varied, but overall there was no significant difference between sexes. Similar to the pre-nesting period, additional data are necessary to determine if differences in incubation are due to normal individual variation or if they are related to mercury exposure.

In the post-nesting period (chick between 1-35 days), no noticeable differences were evident between sexes in parental care of young or other behaviors. In this study, males and females spent a significantly greater percentage of time caring for the young than other maintenance behaviors than in the Midwest. However, our sampling may be biased toward adults tending the young.

Behavioral relationships with Hg risk

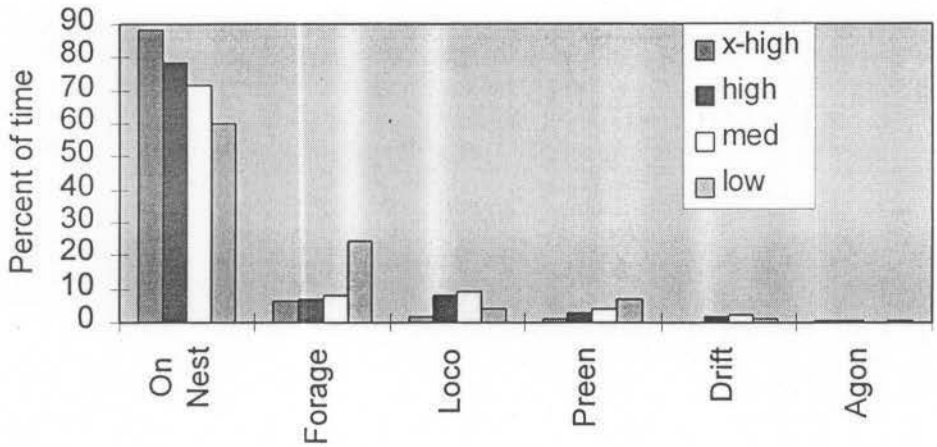
Within our study, territories were divided in four mercury exposure categories: extra high, high, medium and low (Table 1). We compared the mean percent of time spent in each behavior among these categories to determine behavioral differences that might be associated with mercury. Because sample size of TABs were relatively low and mercury exposure values were not complete for each territory, we did not perform linear regressions or multivariate analysis to isolate behavioral effects attributable directly to mercury exposure. However, we have isolated behavioral differences among groupings of territories based on mercury exposure categories.

During the nesting period, adult loons spent a greater proportion of time incubating eggs in higher Hg exposure categories (Figure 3). Adults in lower Hg exposure categories spent a greater



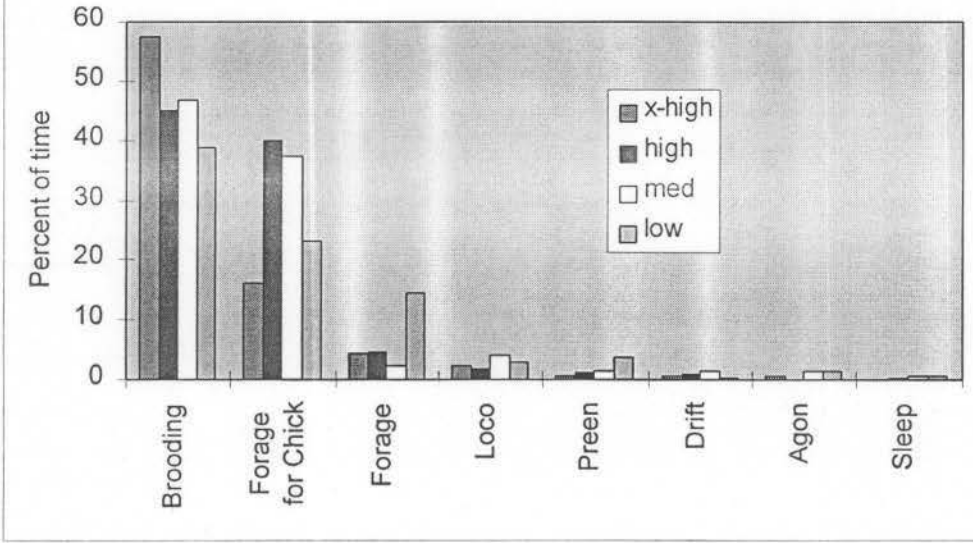
percentage of time foraging and preening. Reasons for differences in incubation, foraging and preening are unclear and warrant further investigation. Interspecific (agonistic) behavior was not notable, different among mercury exposure categories.

Figure 3. Average percent of time adult Common Loons (M&F) spent in 6 behavioral categories during nesting in 4 mercury exposure categories



Behaviors of adults tending young, downy chicks (0-14 days old) were also compared among the four Hg exposure categories. Adults in the extra high category spent less time foraging for their chicks, but more time brooding (directly caring for the young) (Figure 4). Because these adults are spending less time feeding their young, chick growth rates and survival could be affected.

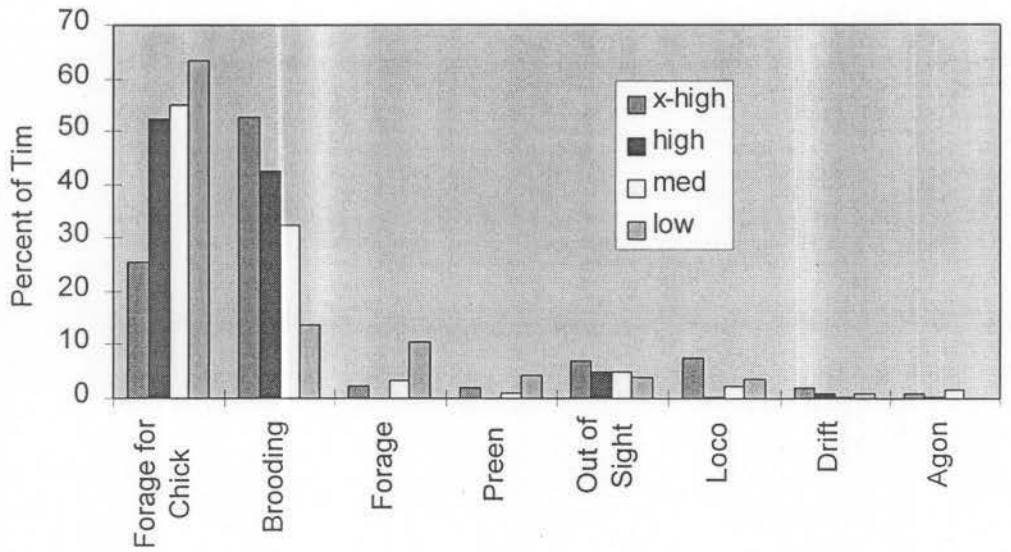
Figure 4. Average percent of time adult Common Loons (M & F) spent tending 0-14 day old chicks in 4 mercury exposure categories



Adults in higher categories also spent less time in self maintenance behaviors such as foraging and preening. Two of the extra high Hg exposure territories had isolated nesting areas, not visible from the rest of the territory. Behavioral sampling from these territories may be biased towards adult tending young and results may be due to insufficient sample sizes. Future sampling of adults in high exposure categories should contain representative samples of adults tending young and those involved in self maintenance behaviors.

Adults tending older chicks (3-5 weeks old) in the extra high Hg exposure territories spent less time foraging for their chicks (Figure 5). We also found a corresponding decrease in the amount of time adults spent brooding as mercury exposure decreased. Because these adults are spending less time feeding their older offspring (consistent with our findings with downy young), chick survival could be affected. Loons from low mercury exposure territories spent more time foraging and preening while extra high loons spent more time at locomotion and drifting than in other exposure categories. Samples sizes are insufficient to determine whether behaviors are solely related to mercury.

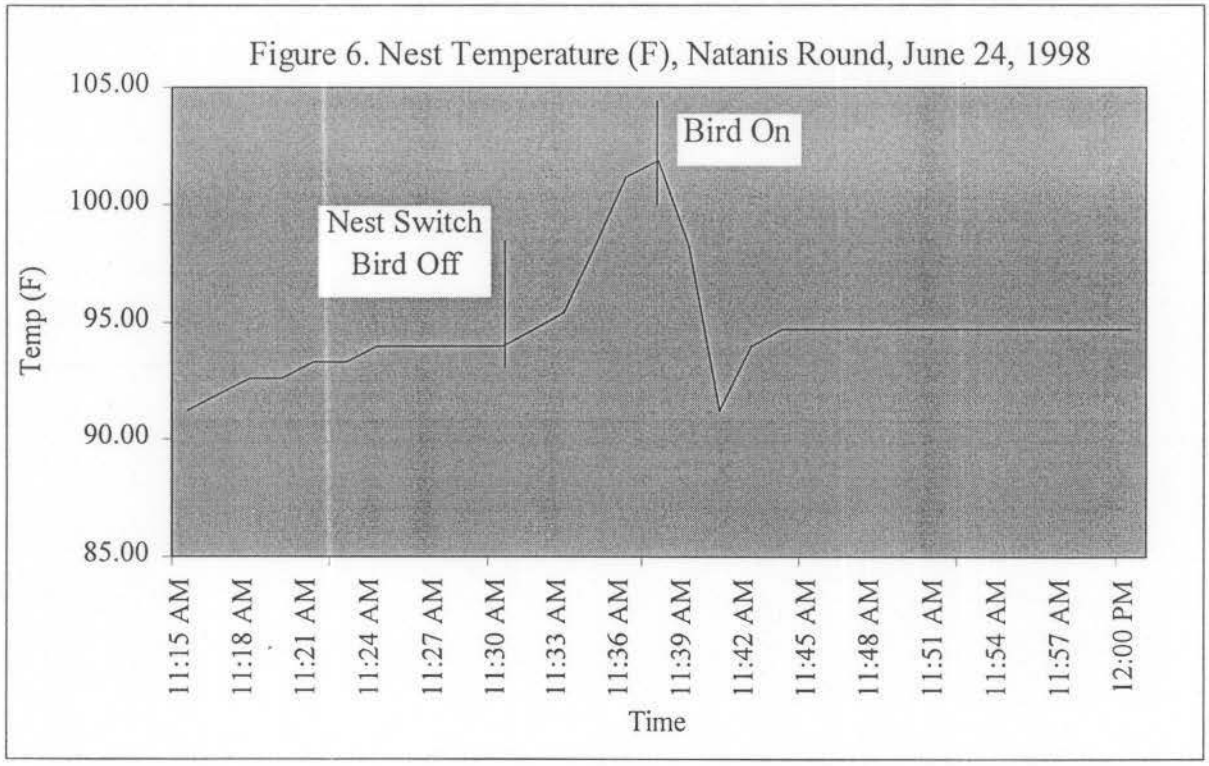
Figure 5. Average percent of time adult Common Loons (M&F) spent in 8 behavioral categories while tending 15-35 day old chicks in 4 mercury categories



While these results do not definitively show that mercury is affecting adult loon behaviors, we have documented lower adult foraging rates for young and a lower percentage of maintenance behaviors in loons inhabiting higher mercury territories. During the pre-nesting period we had sufficient sample size to compare only the medium and extra high territories and found no notable differences in behaviors of territorial adults. Future adult behavioral data should focus on collecting sufficient samples in all Hg exposure categories and increasing capture efforts in juveniles and adults to allow more rigorous statistical analyses. Preliminary evidence of adult behaviors during nesting and post-nesting periods indicates that mercury may be affecting behaviors. Adverse alteration of these behaviors could affect individual adult performance, chick survival and adult survival.

Temperature Dataloggers as measures of adult incubating behavior

Temperature data loggers were placed in twelve different nests during 1998, for a total of over 1,600 hours of monitoring. Both nest and ambient temperatures were monitored on 8 of the 12 sites. Using overlapping TABs, nest switches, egg turns, nocturnal and diurnal nest and ambient temperatures, and flushings off of nests due to human or predatory disturbance were recorded. A clear example of nest switching as indicated by the data loggers occurred on Round Pond (in the Chain of Ponds) on June 24 (Figure 6).

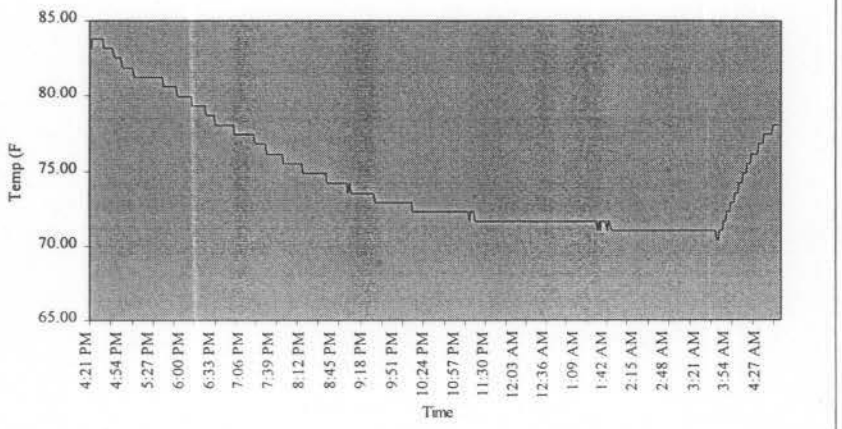


The nest temperature rose after the loon left the nest, as the nest was in direct sunlight. The nest temperature then declined seven minutes later due to the water and increased aeration of egg turning associated with the arrival of the loon's mate.

The temperature data logger on Round Pond also captured the effect of human disturbance on nesting behavior. A nesting loon flushed at 4:20 pm on June 23 and did not incubate until approximately 3:40 am the next morning. This time away from the nest is very unusual and may have been due to constant human activity in the late evening (Figure 7).

An average of the daily nest temperature (7 am to 10 pm) of 8 data sites was 81.04° F, with a correlative average ambient temperature of 67.56° F. Average nighttime (10 pm to 7 am) nest temperature was 77.35° F, with a correlative ambient temperature of 59.16° F.

Figure 7. Nest Temperature (F), Natanis Round, June 23-24, 1998



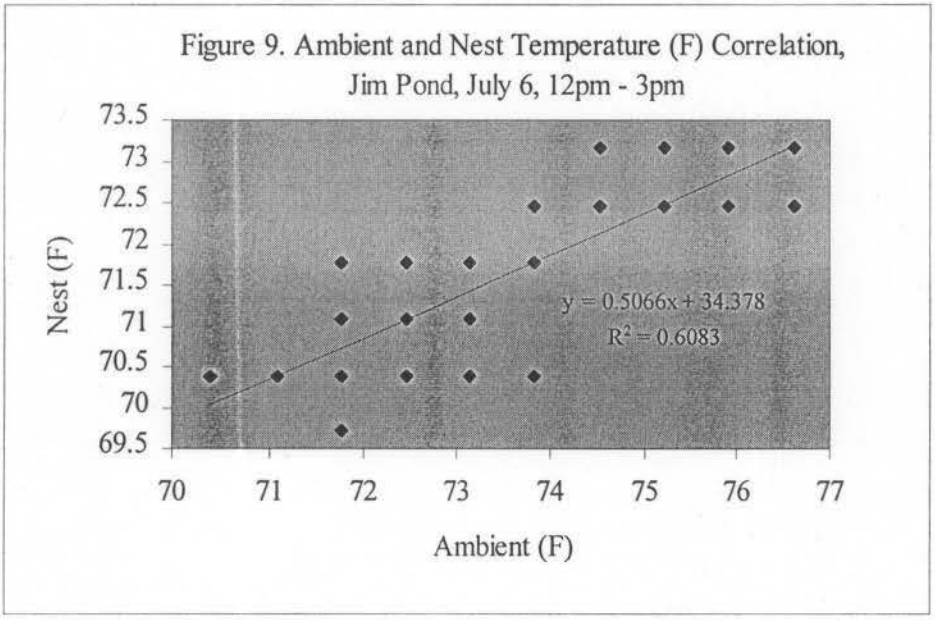
A significant, positive correlation between daily and nightly ambient and nest temperatures was found (Figures 8-9). While no significant differences between high-level mercury lakes (as determined via the loon risk to mercury exposure) and low-level mercury lakes was determined, several nights recorded on moderate to high-level mercury lakes showed inverse relationships between ambient and nest temperatures. These relationships might indicate possible high-stress periods and need to be studied further. An analysis of diurnal and nocturnal temperatures on 8 lakes indicates high-level mercury lakes may have a higher disparity of daily nest temperatures. Initial data (n=3) show moderate-level mercury lakes have an average of 2.09° F difference between diurnal and nocturnal nest temperatures. High to extra-high-level mercury lakes (n=3) have a daily nest temperature fluctuation of 4.43° F.

Figure 8. Ambient and Nest Temperature (F) Correlation, Little Beaver, July 9, 12am - 3am



While data from 1998 provide confidence in the use of temperature data loggers to monitor nesting behavior, more data are required to define the data loggers' sensitivity. Objectives for 1999 include:

- increase the sample size of TABs conducted on data logger sites with high and low mercury individuals as measured in feathers,
- target high and low mercury sites, as measured in adult loon blood and feathers, to further address questions of diurnal and nocturnal temperature variations, and relationships between nest and ambient temperatures.

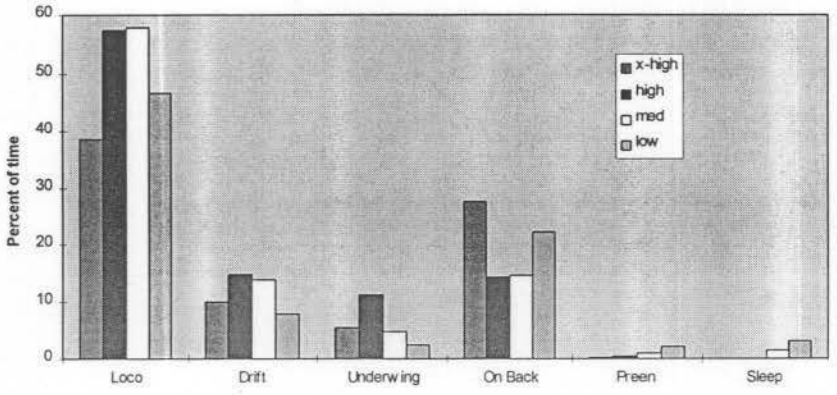


4. Impacts on Chick Behavior

Nocera and Taylor (1998) found a significant negative correlation between blood Hg levels in juvenile Common Loons and back riding and a positive correlation with preening. They postulated that chick survival might be affected by increased rates of predation as a result of not being on the adult's back and less time spent foraging. In this study we recorded juvenile loon behaviors in the 0-14 and 15-35 day old age categories with the same methodologies used by Nocera and Taylor for comparability.

No notable differences were recorded in downy chick (0-14 days) back riding among different mercury exposure categories (Figure 10). Juveniles from lower mercury territories spent more time preening and less time under the wing of adults. They also spent more time sleeping. The significance of these findings is uncertain.

Figure 10. Average percent of time spent in 6 behavioral categories of 0-14 day old Common Loon chicks for 4 mercury exposure categories



Likewise we did not find any major differences in back-riding or preening in 15-35 day old chicks among categories. Juvenile loons in extra high mercury categories did spend less time drifting than lower mercury categories and more time swimming. This may be related to lower frequency of feedings from their parents (Figure 4 and 5).

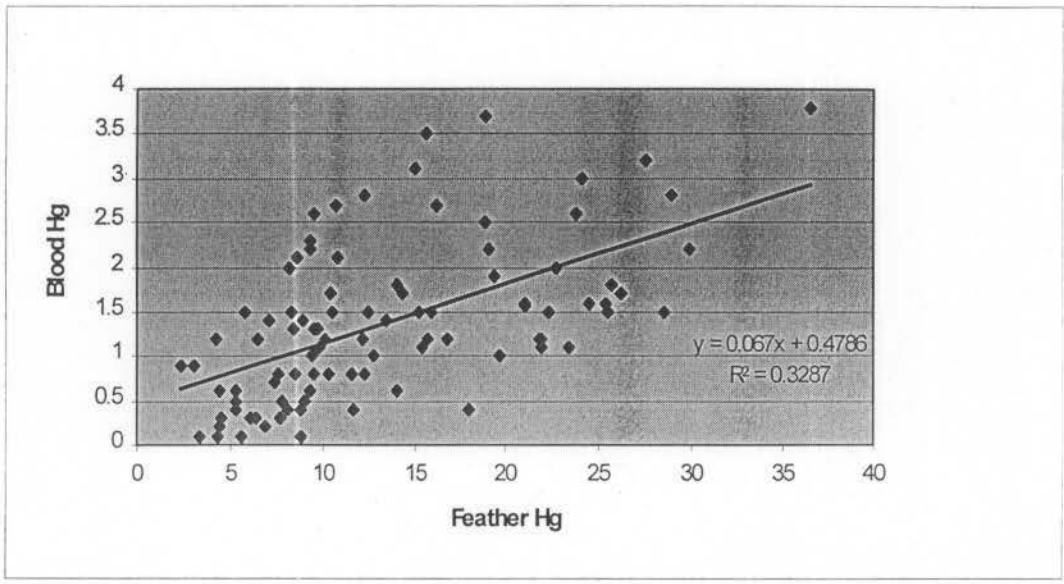
Additional data is required before correlations between mercury exposure and behaviors are examined. Preliminary evidence suggests that juvenile behaviors in high and extra high mercury territories are not adversely affected by Hg exposure. Future analyses will include more subtle behavioral events (behaviors lasting less than 20 seconds) and subcategories of behaviors (e.g., behaviors of juveniles while back riding). Larger sample sizes are needed for this resolution of analyses.

5. Potential Impacts on Individual Survival

Adults and juveniles show increasing levels of Hg in their body burdens over time (Evers et al. 1998a, BRI unpubl. data). Although birds have natural defense mechanisms for depurating (e.g., feathers), demethylating (e.g., liver and kidney), and sequestering (e.g., egg) mercury (Thompson 1998), high risk individuals accumulate more Hg than they are able to annually regulate. Excess Hg binds to protein in the muscle tissue and remobilizes during stressful events. Feather molts are energetically demanding, particularly the full remigial molts that loon's experience for two weeks during the winter. Because muscle protein reservoirs are associated with feather protein (Murphy 1996), the remobilization of proteins during feather molt partly reflect the available body burden of MeHg in an individual loon. We found a significant part of this muscle-bound MeHg originated from prey during the breeding season. There was a significantly positive relationship between blood and feather Hg levels (r^2=0.32, $p<0.01$) (Figure 11).

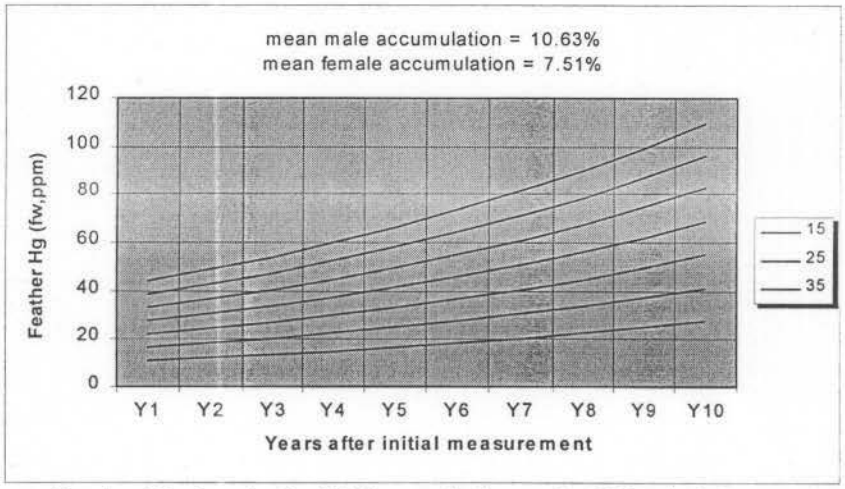
Therefore, the feathers grown during the winter and sampled during the breeding season are indicators of chronic body burdens of Hg. Feather samples collected from recaptured adult loons now indicate this bioaccumulation of Hg is measurable over time.

Figure 11. Relationship between blood and feather Hg levels (ppm) in New England Common Loons



Adult loons were recaptured over the past 1-4 years from Maine lakes. Of 9 males representing 18 accumulation-years, 17 (94%) showed an increase. The mean annual accumulation rate for males was 10.6%. Of 5 females that were recaptured, representing 21 accumulation-years, 18 (86%) showed an increase. The mean annual accumulation rate for females was 7.5%. Male accumulation rates were most likely higher than females because of the females' ability to sequester Hg in eggs (Kambamandi-Dimou et al. 1991) and tendency to eat smaller prey items than males (Evers and Reaman 1998). Male loon body mass averages 23% larger than females in New England.

Figure 12. Bioaccumulation of Hg measured in feathers of recaptured adult loons



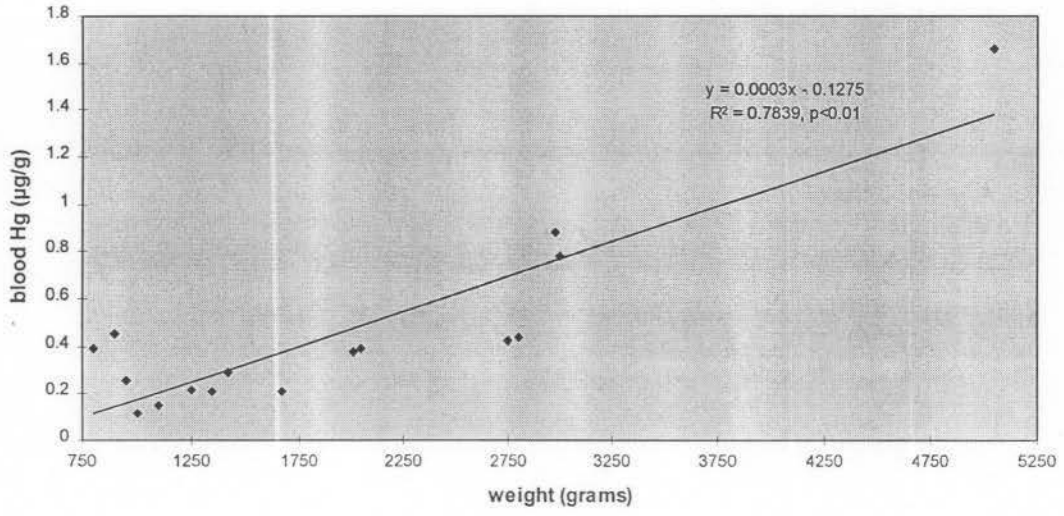
The mean feather Hg levels for Maine male loons is 17.2 +/- 8.7 ppm (n=68) and for females is 12.8 +/- 5.8 ppm (n=71). A temporal extrapolation using an accumulation rate of 10.6% for a male with 15ppm of Hg in its feather places that individual at high risk and potential impacts in 3

years (i.e., 20 ppm) and probable impacts (i.e., 35 ppm) in 7 years (Figure 12). Although female bioaccumulation rates of Hg tend to be lower ($p>0.5$) this rate still only increases the female's reproductive expectancy one year. Because individual loons are K-selected species, long-term impacts on their reproductive success can potentially have severe population effects.

Although environmental Hg levels are known to impact bird embryos, there are also potential impacts on juvenile growth rates and survival. Once a juvenile loon has fully grown feathers, its body burden of Hg can no longer be depurated and instead builds up in its blood, brain, organs, and muscle (Frederick et al. 1997). Young loons have three feather molts before attaining a juvenile plumage. From hatching to 2 weeks a black downy plumage covers the chick's body that is replaced by another downy, but brown plumage lasting for 3-4 weeks. At 6 weeks many of the contour feathers have replaced the downy plumage which can only be found on the nape, neck, and flanks. Flight feathers begin to sheath at 4 weeks and are fully sheathed by 12 weeks. Rate of primary growth is consistent from 5 to 11 weeks, while overall growth rates begin to fall at 10 weeks (Barr 1996).

We captured 3 to 12 week old loons on Flagstaff and Aziscohos Lakes from 1994-98 and found a strong, significant correlation ($p<0.01$) between blood Hg concentrations and weight (Figure 13). Although loon weights can vary with nutritional and physical stress, we used weight as an indicator of age. Therefore, as young loons aged on their natal territories, Hg levels significantly increased ($p<0.01$). Juvenile loons from lakes with lower risk to MeHg availability did exhibit significant increases but were more weakly correlated with weight (i.e., age).

Figure 13. Blood Hg concentrations versus weight in juvenile Common Loons from Aziscohos and Flagstaff, 1994-97



6. *Biomarkers*

The measurement of various blood chemistry parameters and hormones provide a way for assessing an organism's well-being. They also can be used as biomarkers that can demonstrate the presence and extent of contaminant exposure to an organism and predict potential impacts on that individual (Bensen et al. 1990). For example, Frederick et al. (1997) found a relationships of decreased packed cell volume (PCV) with elevated Hg levels in dosed egrets. Colburn et al. (1996)

identified Hg as an endocrine-disrupter and since the loon's body burden of Hg nears the highest measured levels for wildlife in freshwater systems we measured male estrogen levels. Corticosterone hormones are released during periods of stress and are being increasingly used as indicators of environmental stressors, including Hg (Friedmann et al. 1996).

Blood Profiles

In the past five years, Tufts University and BioDiversity Research Institute have collaborated to develop suitable and logistically simple biomarkers for Hg and to determine reference levels of various hematological parameters in the loon. Over 200 adults and 100 juvenile loons have been sampled. Blood profiles have commonly been used in birds as measures of the impact of Hg on an individual's overall health (Frederick et al. 1997, Hoffman and Heinz 1998, Wolfe and Norman 1998). Statistically significant relationships with some blood parameters have been detected with increasing MeHg levels (Frederick et al. 1997, Hoffman and Heinz 1998).

We measured packed cell volume, number and type of white blood cells, and plasma protein. We did not find a significant relationship between elevated Hg concentrations and PCVs ($p>0.5$), white blood cell abundance ($p>0.5$), or white blood cell types ($p>0.05$). Other studies found relationships in birds (all dosed in captive situations) with Hg levels higher than those found in our wild loon populations (Frederick et al. 1997). However, the lower variability in white blood cells for loons with higher levels of Hg suggests further sampling efforts of high risk individuals is needed.

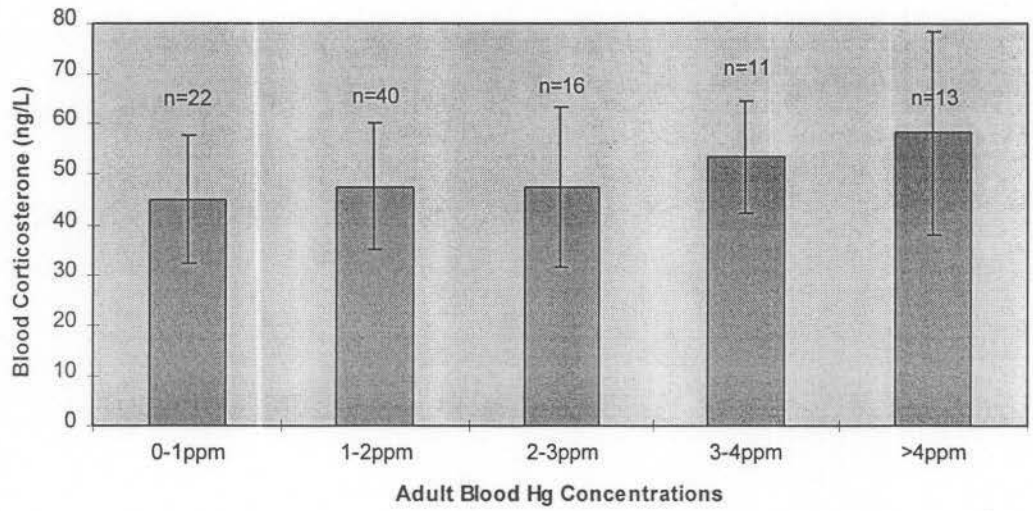
Corticosterone

Although several studies have demonstrated relationships with Hg levels and cortisol stress hormones (Friedmann et al. 1996, Hontela et al. 1992, 1995) those relationships have not been found in birds. BRI collaborators at Tufts University School of Veterinary Medicine have recently developed radioimmunoassays for measuring corticosterone levels in loons. Corticosterone is released in response to stressful stimuli and can potentially provide evidence of immunosuppression. There are many confounding factors when comparing corticosterone and MeHg levels, such as handling stress, reproductive stress, and nutritional stress (Sturkie 1986).

From 1995-1998, we collected plasma from 102 adult loons and analyzed the levels of corticosterone. Sturkie (1986) reported ranges of 0.4 to 29 ng/mL for non-stressed birds. We found corticosterone levels to be elevated (i.e., >30 ng/L) in 88% of the individuals, which ranged from 8.2 to 85.2 ng/mL. Our elevated levels are most likely partly attributed to the stress of capture. However, we were able to account for stress-released corticosterone levels attributed to Hg with the 22 loons in the low Hg risk category (0-1 ppm in the blood). When comparing the mean of capture stress-induced corticosterone levels for the low risk category with the means of loons from higher risk categories we found a significant increase ($p=0.05$) for adult loons under extra high risk to Hg levels (blood Hg > 4.0 ppm) and marginally significant increase ($p=0.07$) for high risk loons (blood Hg levels 3-4 ppm) (Figure 14).

Although capture of adult loons with nightlighting methods and the ensuing 30-45 minute handling time appears to initiate a physiological reaction, stress in our loons with the highest body burdens of Hg does not solely reflect our capture impacts. Further sampling in the high and extra high risk group is deemed a high priority.

Figure 14. Mean concentration of corticosterone versus blood Hg concentrations in adult Common Loons from selected sites in North America (bars represent +/- 1 SD)

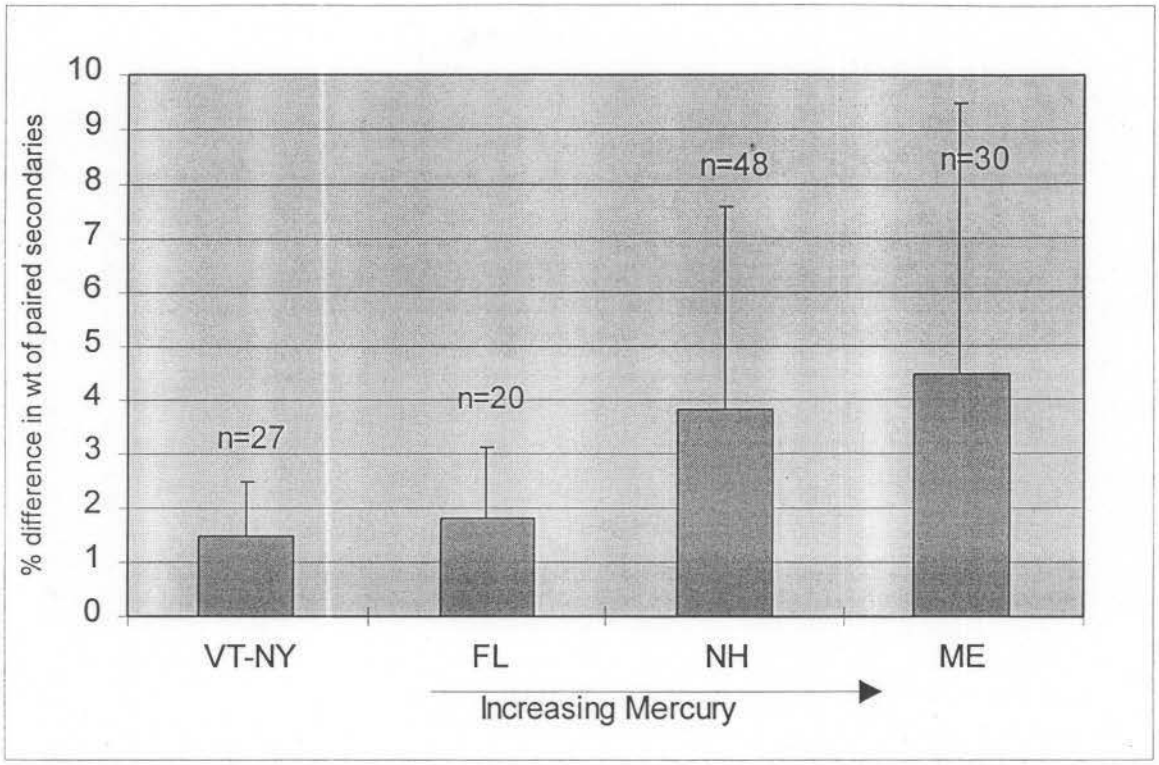


Developmental Stability

We measured the relationship of lifetime Hg body burden and fluctuating asymmetry (FA). Clarke (1995) considered the ability of an individual to develop bilateral characters to be one of the best estimates of developmental stability—an indirect measure of fitness. Because feather growth is linked with the very protein reserves that are associated with bound-MeHg in the muscle tissue (Murphy 1996, Scheuhammer 1991), it is likely that remobilization of MeHg coincides with the proteins used for feather formation. Clarke (1992,1995) and Polak and Trivers (1994) suggested fluctuating asymmetry to be sensitive measure of long-term body condition and Yablokov (1986) and Moller and Swaddle (1997) both considered FA as a sentinel for subtle environmental perturbations prior to visible effects in population viability.

Analysis of 125 paired feathers collected and measured in 1998 indicate a tendency for breeding populations with higher mean feather Hg concentrations to be more asymmetrical (Figure 15). We measured differences in the weight and length of paired second secondaries and compared with feather Hg levels. Because weights had less of a measurement error than length, although they were strongly correlated (r^2=0.86), we used differences of paired feather weights as measures of fluctuating asymmetry. Differences in paired feather weights were not significantly correlated with feather Hg levels ($p>0.05$), however, when we pooled individuals according to state as an indicator of Hg stress to breeding populations, New England (Maine and New Hampshire) breeding adults had significantly more asymmetrical second secondaries than breeding populations with significantly lower feather Hg levels ($p<0.05$). Feather samples from Florida most likely represent breeding adults from the upper Great Lakes (Evers et al. 1999) which also have mean blood Hg levels significantly lower than New England loons (Evers et al. 1998a).

Figure 15. Geographic differences in developmental stability measured through fluctuating asymmetry of Common Loon second secondaries.



It appears that the loon's remiges are a sensitive indicator of FA and the relationship of FA with high Hg risk breeding loon populations potentially makes this bioassay technique important for monitoring aquatic integrity. Although other stressors may disrupt developmental homeostasis, and genetic diversity (especially in the loon, e.g., Dhar et al. 1997) may predispose some populations to have greater FA than others, this technique should be viewed as an excellent "catch-all" benchmark for predicting subtle environmental stressors.

Using mercury impact levels for risk assessment

The sustainability of North America's aquatic landscape depends on minimizing anthropogenic impacts. In recognition of these potential threats, the United States Environmental Protection Agency (EPA) uses the Environmental Monitoring and Assessment Program (EMAP) as a long-term tool for monitoring and assessing ecological condition (e.g., effectiveness of the Clean Air Act). The monitoring of surface waters using EMAP's probability-based surveys for ecological indicators provides a statistically valid technique for making regional and eventually national extrapolations of the exposure and effects of various environmental stressors (e.g., Whittier et al. 1997, Yeardley et al. 1998). The complementary regional program, REMAP, has also proven effective in this same regard (e.g., Stafford and Haines 1996, Mower et al. 1997). EMAP and REMAP efforts within Region 1 and elsewhere are designed to provide a method for evaluating and prioritizing the threat of environmental stressors to lacustrine habitats. One issue that has surfaced from these studies is the widespread and elevated levels of Hg in sediments, water column, fish tissue, and piscivorous wildlife.

Because lacustrine habitats are endpoints of deposition within their watershed they serve as natural collectors of anthropogenic pollutants within the landscape and serve as ideal ecosystems for

select ecological indicators. Lakes, reservoirs, and ponds also attract intense public recreational use and development.

Although biotic sampling from 1993-98 indicates geographic differences in MeHg availability is primarily related to hydrological and biogeochemical factors across the northern part of Region 1 (Evers et al. 1998b), there are areas with elevated Hg concentrations more likely related to point sources. Site specific criteria need to be established in these higher risk areas to determine the extent of point source impacts and to monitor temporal changes in the deposition of Hg. A geographic risk assessment using EMAP/REMAP protocols needs to incorporate and characterize unique sites.

We recommend further studies to continue using selected piscivorous birds as ecological indicators of aquatic integrity for multiple geographic and ecological scales in the Northeast United States. Promising objectives include:

a. Assess the feasibility of using a piscivorous bird guild as a multi-scale ecological indicator of environmental stressors using mercury as a case study
b. Develop site specific criteria for mercury contamination that may be used in models across multiple geographic and ecological scales
c. Use U.S. EPA Environmental Monitoring and Assessment Program protocols to spatially construct regional risk assessments for methylmercury availability in Maine.

Publicity

"Loons tested for lead, mercury," in The Citizen, Laconia, N.H., July 31, 1998.
"Loons captured—for own good," in Concord Monitor, Concord, N.H., August 3, 1998.
"Mercury crisis could be averted," in Sun-Journal Sunday, Lewiston, ME, May 1, 1998 (copy enclosed).
"Night with the loons reflects God's glory," in The Union Leader, Manchester, N.H., August 8, 1998.
"The silence of the loons," in the Boston Sunday Globe, August 2, 1998.
"Loon sampling," in The Meredith News, Meredith, N.H., August 5, 1998.
"Aziscohos lake mercury level to be studied," in The Sun Journal, Lewiston, ME, May 14, 1998 (copy enclosed).
"Loons of Maine," episode of the television program, ALL BIRD TV, aired on the Animal Planet Channel, Fall 1998.
"Mercury rising," in Earthwatch: The Journal of the Earthwatch Institute, September, 1998 (copy enclosed).
"Maine's loon population and mercury," television segment on ABC Channel 8, WMTW, Nightly News.
"Loons of Maine," episode of The Maine Outdoorsman, Ursus Productions, aired December 6, 1998.
"Loons of Maine," episode of Northeast Outdoors, Ursus Productions, aired October 11, 1998.
"Mercury discharge in Maine," episode of the Maine Watch Show, Maine Public Television.

The following are presentations incorporating information from this study:

"Assessing the risk of mercury in a breeding population of Common Loons in New England," at the regional mercury conference in Fredrickton, New Brunswick, November, 1998.
"Understanding the impacts of mercury to New England's Common Loon," at the Earthwatch Institute conference in Boston, MA, November, 1998.
"Maine's breeding loons are at risk to mercury," at the Maine Bird Conference, Auburn, Maine, 1998.
"Effects of mercury on wildlife," at the Conference of New England Governors and Eastern Canadian Premiers, in Portland, Maine, 1998

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Many individuals, organizations and agencies assisted with this study. BRI staff included Shearon Clarke, Paula Shannon, Matt Munson and Oksana Lane. They all put in long hard hours and were instrumental to this project's success. Thirty volunteers from the Earthwatch Institute were valuable assistants with the behavioral studies. Thanks to Kate Taylor and other Loon Preservation Committee staff, for preparing meals for the summer that were always delicious. We also thank Drew Major and Ken Munney of the USFWS and Barry Mower of the Maine DEP for logical support. Kate Taylor and Lee Attix from LPC assisted with egg collections and processing, capture of loons, behavioral observations, and overall productivity information from New Hampshire lakes. Mark Pokras and Rose Miconi supervised hormone assays and Holly Haefle determined blood parameters. Bob Poppenga of University of Pennsylvania supervised lab analysis for Hg in eggs, blood and feathers. Financial subsidies from UPenn greatly assisted in increasing tissue sample sizes. Bill Hanson, John Perry and Kyle Murphy of Central Maine Power Company assisted with loon capture and provided equipment throughout the summer. Thanks to the Marshall Swain family in Rangeley for the use of boats and assistance in capture efforts. Thanks also to Carrie King for assistance in statistical analyses of egg parameters. Dave Yates assisted with the preparation of this report. We thank Don McNeil for assisting with TABs on Flagstaff. This study was integrated into the workscope of the Northeast Loon Study Working Group (NELSWG), a coalition of state and federal agency representatives, universities, non-profit organizations and other interested parties and members. We thank all members of NELSWG who contributed their expertise and enthusiasm to this study.

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Appendix 1

BEHAVIOR STATE AND EVENT PROTOCOL

Guidelines for Identifying Behavioral States and Events

Changes from one state to another only occur after 20 seconds into the new behavior with the exception of **Foraging**, in which 2 minutes must pass before recording new state (see exception below). Record the minute and second that each state begins and ends. Events are primarily behaviors that last less than 5 seconds and are not timed. They are tallied (counted) on the data form during the different behavioral states. When recording behaviors in loon territories chick behaviors (states and events) always take priority over adults. States (*S*) and Events (*E*) are indicated next to the behaviors below. In some cases, a behavior can be both a state and event depending on its duration (< or > than 20 seconds).

ADULT–SPECIFIC BEHAVIORS

Courtship: *S* - Formalized and synchronous bill-dipping
Nest Building: *S* - Collection and placement of nest material
Nest Sitting: *S* - Anytime an individual is on nest incubating eggs. Use the following subcategories to characterize this behavioral state:
- **Content:** *S* - In a normal, low energy behavior
- **Alert Posture**: *S* - Neck extended, usually in response to an intruder
- **Sprawl:** *S* - Low profile not in response to intruders but usually due to heat.
- **Hunkered:** *S* - Low profile in response to intruders.
- **Egg Turning:** *S&E* - Sits up and turns eggs with its bill
- **Panting:** *S&E* - Bill open
- **Tending:** *S&E* - Movement of nest material, separate from egg turning

Territorial Encountering (Agonistic): *S* - Interactions with con or interspecifics within the loon's territory. Characterized by circle swimming, bill dipping, splash diving, alert posture and oaring (see Intrusion form to fill out more information). ENCOUNTERING BEGINS WHEN TERRITORIAL BIRDS ARE WITHIN 25 METERS OF INTRUDER (S).
Penguin Dancing (PD): *E* - Body vertical in water with wings tucked in close to body, bill pointed straight ahead or down. This is a very aggressive behavior and is in response to an intruder.
Rushing (R): *E* - Using wings and feet to move over surface of water
Brooding: *S* - One or both adults closely attending one or both young (within 20 feet). Use the following subcategories to characterize this behavioral state:
- **Back Riding:** *S* - 1 or 2 chicks on the back of the adult (if more than one chick record behavior of each)
- **Under Wing:** *S* - 1 or 2 chicks under the wing of the adult
(if more than one chick record behavior of each one)
- **Brood-swim:** *S* - Brooding adult swimming and chick(s) in water
- **Brood-drift:** *S* - Brooding adult drifting and chick(s) in water
- **Brood-preen:** *S* - Brooding adult preening and chick(s) in water
- Note: note time you suspect subcategory state changed during brooding then write down the time when confirmed (20 second rule applies).

Chick Feeding (CF): *S&E* - Adult(s) present food items to chick(s)

ADULT AND CHICK–SPECIFIC BEHAVIORS

Foraging: *S* - Under water feeding (usually <45 seconds) and above water peering and swallowing. Foraging continues for 120 seconds (2 minutes) after the time of the last diving event, THE EXCEPTION IS PREENING FOLLOWING FORAGING, IN WHICH THE 20 SECOND RULE APPLIES
Locomotion: *S* - Expenditure of energy while swimming above and below water. Underwater locomotion only occurs in response to an intruder. Loons may not be moving when they are swimming against the current or into the wind.

Drifting: *S* - No expenditure of energy while at the water surface. Loons may be moving with a current or wind energy with no expenditure of energy. **Sleeping**-head tuck.
Preening: *S&E* - Maintenance of feathers by spreading oil from the uropygial gland.
Bathing: *S* - Extreme preening behaviors that includes erratic behavior such as flipping upside down.
Foot Waggle (FW): *E* - Anytime foot is above water and shaken. Do not record foot extension while preening or during head scratching.
Head Scratch (HSc): *E* - Foot used for scratching head (**do not record while preening**).
Wing Flap (WF): *E* - Body raised and wings outstretched
Alert Posture (AP): Sitting high in water with neck stretched up / held high
Splash Dive (SD): *E* - Strong kick upward while diving causing water to splash, usually in response to an intrusion.
Peering (P): *E* - Head in water up to or over the eyes with body on the surface. This is different than bill-dipping.
Head Rub (HR): *E* - Roll top of head along (**do not record while preening**)
Vocalizing (V): *E* - Record calls, usually wail, tremolo, hoot, yodel, and mew but can be a combination of all the above.
Yawning (Y): *E* - Raising and extending neck leading to opening and closing of bill as it points skyward.
Bill Dipping (BD): *E* - Rapid immersion of the bill tip into the water followed by a rapid flick of the bill to one side (**do not record while preening**)
Head Shake (HS): *E* - Movement of head back and forth at least twice. Note if this is in response to insects.
Panting (PA): *E* - Adult with bill open and panting bout lasts less than 5 seconds.

CHICK-SPECIFIC BEHAVIORS

In two chick families the priority is to record the behavior of each chick seperately, if this becomes difficult (for example they are both foraging) drop the events on one or both individuals

Underwing: *S* - Chick is under the wing of one of its parents (record if the body of the chick is under the wing)
On Back: *S* - Chick is on the back of the adult.
I = *E* - independent boarding - record if chick gets on the parent without help
A = *E* - assisted boarding - record if parent lifts wing to help chick on the back
Begging (B): *S&E* - Chick swimming close to adult, wanting food.
Fighting: *S&E* - Chicks are fighting (pecking, rushing, etc.); they must both be in the water
Stashed: *S* - Chicks are left near shore while parents are engaging a intruder or threat.
Stashed out of sight: *S* - record time you cannot see chicks
Stashed in sight: *S* - record time you can observe chick (behavior is recorded under Stashed state)