Adverse effects from environmental mercury loads on breeding common loons

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Accepted: 13 August 2007/Published online: 2 October 2007 © Springer Science+Business Media, LLC 2007

Abstract Anthropogenic inputs of mercury (Hg) into the environment have significantly increased in the past century. Concurrently, the availability of methylmercury (MeHg) in aquatic systems has increased to levels posing risks to ecological and human health. We use the common loon (Gavia *immer*) as an upper trophic level bioindicator of aquatic Hg toxicity in freshwater lakes. Multiple endpoints were selected to measure potential negative impacts from MeHg body burdens on behavior, physiology, survival and reproductive success. A robust spatio-temporal dataset was used that included nearly 5,500 loon Hg measurements over an 18-year period. We measured significant changes related to elevated MeHg body burdens, including aberrant incubation behavior, lethargy, and wing area asymmetry. Mercury body burdens in adult loons increased an average of 8.4% per year. Increasing Hg body burdens reduced the number of fledged chicks per territorial pair, with highest risk loons producing 41% fewer fledged young than our reference group. Our multiple endpoints establish adverse effect

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M. S. Bank Department of Environmental Health, Harvard University, Boston, MA, USA thresholds for adult loons at 3.0 ug/g (wet weight) in blood and 40.0 ug/g (fresh weight) in feathers. Mercury contamination in parts of Maine and New Hampshire is a driving stressor for creating breeding population sinks. Standardized monitoring programs are needed to determine if population sinks occur elsewhere and to track aquatic ecosystem responses to changes in Hg emissions and deposition.

Keywords Mercury \cdot Common loon \cdot Population sink \cdot Adverse effects \cdot Behavior

Introduction

Mercury (Hg) toxicity to wildlife is widely documented (Thompson 1996; Wolfe et al. 1998; Scheuhammer et al. 2007). The atmospheric deposition of Hg is an important contributor to declining local (Frederick et al. 2004) and global bird populations (Braune et al. 2006). Waterborne

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J. Fair Fairwinds Wildlife Services, Palmer, AK, USA Hg from legacy sources such as mining (Schwarzbach et al. 2006), chlor-alkali plants (Barr 1986), and manufacturing facilities (Brasso and Cristol, this issue) have also been shown to negatively impact breeding success. However, there are few studies that have directly related adverse impacts from Hg on reproductive success in free-living bird populations. Long-lived obligate piscivores such as the common loon (*Gavia immer*) are considered important bioindicators of environmental Hg loads in North America (Evers 2006; Wolfe et al. 2007), because of the ability of methylmercury (MeHg) to biomagnify through the food-chain (Cabana and Rasmussen 1994; Atwell et al. 1998; Burgess and Hobson 2006) and bioaccumulate over an individual's lifetime (Evers et al. 1998).

Numerous studies of Hg concentrations in loon blood, feathers (Meyer et al. 1998; Evers et al. 1998; Burgess et al. 2005; Champoux et al. 2006) and eggs (Scheuhammer et al. 2001; Evers et al. 2003) provide a robust geographic Hg exposure profile. These and other studies indicate loons breeding in northeastern North America have the greatest risk of adverse effects from Hg due to elevated Hg exposure associated with relatively high rural atmospheric deposition (up to 32 ug/m² per year of wet and dry deposition) (Miller et al. 2005) and enhanced Hg methylation in sensitive environments (Driscoll et al. 2007). Biological Hg hotspots have been identified in New York, New England, and Nova Scotia where concentrations are high enough to result in population-level impacts to breeding loons (Evers et al. 2007).

Substantial evidence exists for adverse neurological, physiological, and reproductive effects to loons associated with environmental Hg levels. Neurotoxic effects of MeHg in free-living loons include reduced back-riding by chicks (Nocera and Taylor 1998) and lowered chickfeeding rates by adults (Counard 2000). Recently, changes in neurotransmitter receptor concentration and other neurochemical effects have been correlated with brain Hg concentrations in wild loons (Scheuhammer et al. this issue). Physiological responses to elevated MeHg levels include reduced diving frequency of free-living loons (because Hg inhibits heme production; Olsen et al. 2000) and the production of smaller eggs in the wild (Evers et al. 2003). Sublethal Hg dosing studies in laboratory conditions indicate that histological, immunological, and biochemical biomarkers are effective for detecting adverse effects on juvenile piscivorous birds (Spalding et al. 2000; Henny et al. 2002; Hoffman et al. 2005), including loons (Kenow et al. 2003, 2007). However, the limitation of low adult loon survival in captivity forces laboratory studies to be limited to juvenile loons, an age class characterized by its ability to depurate large amounts of MeHg, especially into growing feathers (Fournier et al. 2002).

Negative impacts of MeHg exposure on reproductive success and survival of adult loons are of ultimate concern. There are inherent difficulties in relating Hg exposure to loon reproductive success that relate to both intrinsic (e.g., density dependence, species longevity) and extrinsic (e.g., weather, habitat quality) factors as well as anthropogenic stressors (e.g., recreational disturbances and other contaminants). Still, there are studies that have identified Hg as a cause of reduced reproductive success in loons. Barr (1986) found whole body Hg levels in prey fish of 0.30 ug/g (ww) resulted in reproductive impairments on breeding loons, and no loon reproduction when Hg in fish exceeded 0.40 ug/g (ww). More recently, Burgess and Meyer (this issue) found a significant negative correlation between Hg in fish and loon productivity with 50% fewer fledged young in breeding loons foraging on fish averaging 0.21 ug/g (ww) and no reproductive success if prey fish exceeded 0.41 ug/g (ww).

In this study, we examine adverse effects of Hg on a free-living breeding population of common loons inhabiting lentic ecosystems in western Maine and New Hampshire using behavioral, physiological, survivorship, and reproductive endpoints based on observational and Hg concentration data over an 18-year period.

Study area

BioDiversity Research Institute (BRI) has collected blood and tissue samples to analyze Hg concentrations in North America's breeding loons annually from 1989 to 2006 (Evers et al. 1998, 2003, 2005). This broad geographic sampling effort has resulted in the testing of 5,477 loon tissues for blood (n = 2,850), feathers (n = 1,589) and eggs (n = 1,038) from 1,086 territories on 701 lakes in 13 states and four Canadian provinces (Fig. 1). The extensive continental dataset was used to examine physiological and survival endpoints and to develop loon Hg equivalent units to support comparisons across tissue type and age and sex classes.

We conducted a high-resolution study on the impacts of Hg on loon behavior and reproductive success in the upper Androscoggin River watershed in New Hampshire and Maine, the upper Kennebec River watershed in Maine, and selected lakes in southeastern New Hampshire based on our extensive historical database. This geographic area had some of the highest Hg levels recorded in North America. Our focal study area included 80 lakes with 178 territories that were regularly surveyed to determine reproductive success from 1996 to 2005 (Fig. 2). The behavioral observations were concentrated on 12 lakes with 43 territories from 1998 to 2000. Fig. 1 General locations of sampling effort for feather Hg levels that correspond with asymmetry and percent change biomarkers. All locations also represent blood and/or egg sampling areas tissues used for developing female loon unit (FLU) equivalents



Sampling efforts in this same geographic area in 1996 to 1997 by the U.S. Fish and Wildlife Service on fish and loons to determine levels of organochlorines and polychlorinated biphenyls (PCBs) found relatively low levels (USFWS, unpublished data). This finding further supported the suitability of this region for studying the effects of Hg on loons and suggested that the results would not be confounded by the potential synergistic effects of organic compounds, such as those known with PCBs (Newland 2002).

Methods

Tissue choice

Three standard biological matrices that could be non-lethally collected (blood, eggs, and feathers) were used to identify adverse effect levels based on pre-existing knowledge of tissue Hg properties for loons and other birds (Evers et al. 2005). Blood is the primary matrix for evaluating recent dietary uptake and there is strong evidence that adult blood Hg levels reflect prey Hg levels in the breeding lake (Evers et al. 2004; Burgess and Hobson 2006; Burgess and Meyer, this issue). Over 95% of the blood Hg is in the methyl form (Wolfe et al. 2007). The half-life of MeHg in the blood for juvenile loons until 12 weeks of age is <3 days and post-molt is 116 days (Fournier et al. 2002). The half-life for MeHg in adult loon blood is unknown; in adult Cory's shearwaters (Calonectris diomedea) it is 40 60 days (Monteiro and Furness 2001). We surmise that adult loon blood Hg levels reflect uptake of dietary Hg from the breeding lake because the blood Hg levels of adult loons returning to their breeding lakes in the spring is likely <1.2 ug/g (based on 105 loons sampled in wintering areas along the Pacific and Atlantic coasts; BRI unpublished data) and adult loons were sampled 2 4 months following their return to lakes.

Asymmetry and Percent Change Biomarker Percent Change Biomarker Only

Kilometers

Asymmetry Biomarker Only 1,500

+

Since female blood Hg levels are highly correlated with egg Hg levels (Evers et al. 2003), eggs are also pertinent tissues for predicting Hg risk within a breeding territory. Egg collection was opportunistic and reflects eggs that were abandoned because of nest inundation by rising water Fig. 2 Locations of common loon territories sampled for Hg and monitored for reproductive success. Major lakes are identified



levels, wave action, human disturbance, storms and other events that force incubating adults from normal incubation behavior.

Feather Hg levels are a useful matrix for evaluating chronic body burdens. Feather Hg can represent 70 93% of the total body burden of Hg (Burger 1993); therefore there can be a chronic bioaccumulation of MeHg because the entire Hg body burden is not depurated each year, particularly for high-risk individuals. Feather Hg reflects blood Hg levels at the time of molt (Bearhop et al. 2000); however, if MeHg is sequestered in the muscle tissue (as is the case for individual birds with a high dietary Hg uptake), then additional MeHg is available through remobilization at a later time. Individual variation in physiological response to Hg (Bearhop et al. 2000), as well as the broad differences of the pharmacokinetics among species, complicates the interpretation of adult feather Hg levels.

Mercury results in tissues are reported as wet weight (ww) in blood and eggs and fresh weight (fw) in feathers.

Capture, marking, and sampling

Capture of adult loons followed night-lighting techniques described by Evers (2001). Blood and feather samples were non-lethally taken. All individuals were banded with a U.S. Fish and Wildlife Service numbered aluminum band along with a unique color combination of custom-formed plastic bands (described in Evers 2001). We attempted to recapture adults to measure changes in Hg body burdens over time in known individuals. High annual territory fidelity rates that average 80% permit effective recapture (Evers 2001).

Tissue collection protocols are well established and described (Evers et al. 1998, 2003, 2005). Blood was drawn from the metatarsal vein and placed in a cooler with ice packs. Whole blood samples were then placed in a freezer within 8 h of collection and not removed until submitted to an analytical lab. Second secondary feathers were symmetrically taken by cutting below the superior umbilicus. They were placed in a polyethylene bag and kept in a cool area. Abandoned eggs were placed in a polyethylene bag and frozen as soon as possible. Later, eggs were measured for standard metrics and placed into sterile I-Chem (R) jars (Evers et al. 2003).

All sample preparation, handling, labeling, and chainof-custody efforts followed well-established standard operating procedures established by BRI.

Loon equivalent development

To best evaluate and utilize existing data from various biotic compartments, Hg concentrations require a single common unit. Accordingly, Hg concentrations in the various loon tissues and age/sex classes (i.e., adult female blood, adult male blood, juvenile blood, and egg) were converted into a female loon unit (FLU) Hg equivalent. Since adult loon weight varied from 3,900 g to 7,550 g, and prey size relates to loon size and Hg levels (Evers et al. 2004), we also accounted for weight within our FLU model. Using our continental dataset, the Hg per weight values for each loon with blood Hg and weight data were calculated and the mean per territory value determined. Using Statgraphics Centurion XV Version 15.2.00, mean blood Hg-weight ratios of females were regressed with mean ratios of males and of juveniles. The optimum male to female relationship was linear; however, the optimum juvenile to female ratio was a double reciprocal. Initially, the regression for juvenile to female levels was poor due to extreme outliers. However, eliminating all values of juveniles for which the Hg to weight ratio for juveniles, divided by that for females was outside the 99.99% confidence interval, improved the regression significantly. Multiplying the regression by the individual's weight converts a male and juvenile blood Hg level, respectively, to a female blood Hg level (i.e., FLU) (Eqs. 1 and 2). Additionally, egg mercury levels (Hgegg) were converted to female levels based on the conversion established in Evers et al. (2003) (Eq. 3).

$$FLU = Hg_{female}$$

$$= [(Hg_{male}/Weight_{male}) \cdot 0.948$$

$$+ 0.000002] Weight_{male}; r^{2} = 0.68 \qquad (1)$$

$$FLU = Hg_{female}$$

= [1/1393.220 + 0.197/(Hg_{juvenile}/
Weight_{juvenile})] Weight_{juvenile}; r^2 = 0.83 (2)

$$FLU = Hg_{female} = Hg_{egg} \cdot 1.554 + 0.224; r^2 = 0.79$$
(3)

Hereafter, we use FLU as our standardized approach for comparison. FLUs are presented on a wet weight (ww) basis as ug Hg/g of tissue.

Behavioral observations

Over a 3 year period, we collected a total of 1,540 h of loon behavioral observations between early May and late August: 1998 (n = 753 h), 1999 (n = 571 h), and 2000 (n = 216 h). Loon territories were placed into three risk categories for interpretive purposes based on Hg concentrations in FLUs: (1) low (<1.0 ug Hg/g), (2) moderate (1.0 3.0 ug Hg/g), and (3) high (>3.0 ug Hg/g). The risk categories were defined based on two thresholds for Hg measurements: (1) in the southern Alaskan loon population, which serves as our low-exposure reference group, all individuals have FLUs below 1.0 ug Hg/g and (2) our results herein document FLUs >3.0 ug Hg/g as having a significant negative adverse effect on reproductive success.

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 because of the strong evidence from Evers (1994), Mager (1995), Gostomski and Evers (1998), and Paruk (2000) that minimal or no significant relationships exist between time of day and loon behaviors.

Individual loons were observed in one-hour time blocks for up to 5 h/day using a 15 45× spotting scope and 10× binoculars. Observers continually monitored behavior through a spotting scope and relayed behaviors to a recorder, who noted times from a digital stopwatch and recorded categorized observations on data sheets. In 1998 and 1999, data were collected by six BRI biologists and trained EarthWatch Institute volunteers. Martin and Bateson (1993) addressed potential problems with observer bias and misinterpretation of behaviors. Therefore, observer bias was minimized each year by training all BRI biologists simultaneously for 3 4 days, and meeting to review methods and results several times throughout the season. EarthWatch Institute volunteers were designated as recorders. 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 distant).

Adult behaviors during post-hatching were designated into two energetically-based categories: (1) high energy behaviors included foraging for chick, foraging for self, locomotion (swimming and flying), preening and agonistic behaviors and (2) low energy behaviors included brooding and resting (drifting and sleeping).

Asymmetry development

To determine feather asymmetry we cut the second secondary at a standard site on the rachis, where the calamus meets the feather vane (i.e., at the superior umbilicus), to standardize measurements of weight. Secondaries were cut on the left and right wing. Measurement precision was 0.001 g using a high-precision digital scale at the University of Southern Maine, Portland, Maine. Differences between left and right wing secondary weights were used for our determination of symmetry. This method is highly replicable and without human bias and therefore offers a high-confidence measurement. Minimizing error and bias in morphometrics measurements is critical for accurately quantifying asymmetry (Helm and Albrecht 2000).

Breeding loon monitoring

From 1996 to 2005, we regularly surveyed territorial loon pairs from early or mid May until late August or early September. We used well-established standardized protocols developed by the Loon Preservation Committee (LPC) (Taylor and Vogel 2000). Surveys consisted of locating territorial pairs every 6 8 days from a boat or shore with 10× binoculars. We collected four reproductive parameters from each territory: (1) presence of territorial pair, (2) nesting attempts, (3) hatching success and (4) fledging success. Successful fledging was defined as a loon reaching age six weeks or older. This definition is consistent with protocols developed by the Northeast Loon Study Working Group (NELSWG) and used by LPC. The unit most representative of overall reproductive success is the number of chicks fledged per territorial pair (CF/TP).

Laboratory analysis

Laboratory protocols for analyzing total Hg follow Evers et al. (2003) for eggs and Evers et al. (1998) for blood and feathers (except for feathers after 2002). Analyses of loon tissues were conducted by the Animal Health Diagnostics Laboratory, University of Pennsylvania, New Bolton, Pennsylvania (for blood and feathers) and the Trace Element Research Laboratory, Texas A&M, College Station, Texas (for blood and egg).

Loon feathers from 2003 to 2005 were analyzed by the Department of Public Health, Harvard University, Cambridge, Massachusetts. Loon feathers were washed three times in acetone, and rinsed in triplicate with deionized water followed by a final acetone wash and air dried in a clean hood (Class 100 Series) for 24 h at Harvard University. After washing, feathers were cut into small pieces using stainless steel scissors (cleaned between samples), homogenized in a cryomill, and weighed in 0.5 mg quartz sample boats. Total Hg was measured in homogenized feather samples (feather sample mass range = $0.001 \ 0.033 \ g$) by thermal decomposition, amalgamation, and atomic absorption spectrophotometry (EPA method 7473; USEPA 1998) using an automated system (DMA-80, Milestone Inc., Monroe, Connecticut, USA). Certified reference materials (CRM; DORM-2, National Research Council Canada, Ottawa, ON, Canada) and procedural blanks were analyzed periodically (every 10 20 samples) to evaluate accuracy and ensure low blanks and minimal instrument drift. Recovery of Hg in CRM was between 94 103% and precision, as measured by randomly selected duplicate samples, averaged 90% (n = 12).

Statistical analysis

Mercury concentrations are expressed as arithmetic means because of generally large datasets that were deemed normally distributed based on normal probability plot residuals and use of the Kolmogorov Smirnov test. Homoscedasticity was checked with Bartlett's test, which is sensitive to the normality assumption. JMP software (SAS Institute 1999) was used to test various hypotheses using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference tests if our ANOVA showed significant differences. Student's t-test was used when comparing paired data sets. JMP software corrected for inequity of unbalanced data sets. In cases where normality or homoscedasticity tests failed, we used nonparametric tests. In all cases, means are given with one standard error (SE) unless otherwise noted. The level of statistical significance was defined as an alpha value of 0.05. Graphs were generated by SigmaPlot ver. 10 (Systat 2006).

Results

Mercury exposure in the focal study area

Blood, egg, and feather Hg levels in the focal Maine New Hampshire study area ranged from levels similar to our Alaskan reference group to some of the highest recorded in North America. Adult blood Hg levels ranged from 0.13 to 11.80 ug/g (ww) with a mean (\pm SE) of 1.73 \pm 0.06 ug/g (n = 644). Egg Hg levels ranged from 0.20 to 9.03 ug/g (ww) with a mean (\pm SE) of 1.63 \pm 0.82 ug/g (n = 366). Feather Hg levels ranged from 1.4 to 75.7 ug/g (fw) with a mean (\pm SE) of 16.7 \pm 0.4 ug/g (n = 302). Adverse effect thresholds were exceeded in 19% of the eggs, 16% of the adult blood, and 3% of the adult feathers.

Behavioral endpoints

We found a significant negative relationship between adult blood Hg levels and the percent time adult male and female loons spent in high energy behaviors while brooding 1 40 day old young (F = 11.6, df = 18, p = 0.003; Fig. 3) that was predictive ($r^2 = 0.41$)

Behavioral data for time on nest did not meet normality and homscedasticity assumptions. A ranking of the three Hg risk categories indicate that high Hg loons spent less time nest sitting than did moderate- and low-risk individuals (n = 152 h). Males and females at low Hg risk spent an average of 99% of the time incubating eggs, leaving the eggs unincubated for only 1% of the time sampled (n = 45 h). Loons in the moderate risk category left eggs



Fig. 3 Average time adult male and female loons spent in high energy behaviors while brooding 1 40 day old chicks related to mean blood Hg levels in Maine, 1998 2000. Semi log plot includes 95% confidence intervals

unincubated for an average of 10% of the time (n = 33 h), and high risk males and females left the eggs unincubated for an average of 14% of the time sampled (n = 74 h).

Physiological endpoint

We used differences of the weights of paired flight feathers as a measure of fluctuating asymmetry (n = 664 paired feathers) and compared them with five groups of Hg levels that were evenly divided. Feather weights were significantly different between the highest Hg group (>40 ug/g) and the lowest Hg group (<10 ug/g) with high Hg adults having greater flight feather asymmetry (t = 1.97, df = 230, p = 0.02) (Fig. 4).



Fig. 4 Developmental stability measured through differences in the mean weight $(\pm SE)$ of common loon secondaries measured symmet rically compared to five feather Hg groups

Survival endpoint

We determined annual changes of feather Hg levels from known individual loons that were recaptured at least one breeding season later. On average, the annual change for adult loons was an increase of 9.2% for males and 7.4% for females (n = 238, 203 resampled individuals, respectively) or 8.4% when sexes were combined (Fig. 5). There was no significant difference between sexes (t = 0.77, df = 439, p = 0.44).

Reproductive endpoint

The reproductive success of 178 territories on 80 lakes was monitored for 6 to 10 years equivalent to 1,529 territoryyears. Summaries of the reproductive success of loons from our study area provide a high-resolution description of annual number of territorial pairs monitored and their nesting, hatching and fledging success (Table 1). When comparing the average CF/TP with FLUs we found a significant negative correlation between fledging production and Hg concentrations (F = 4.04, df = 176, p = 0.04) (Fig. 6). We did not find significant among-year differences in CF/TP (F = 0.92, df = 9, p = 0.51).

Discussion

Significant adverse effects from Hg on breeding loons were determined using behavioral, physiological, survival, and reproductive endpoints. We believe the weight of evidence indicates that population-level effects occur in parts of Maine and New Hampshire, and potentially in broad areas of the loon's range particularly where there is high sulfate deposition (Jeremiason et al. 2006) and other



Fig. 5 Annual percent change of Hg measured in feathers of recaptured adult male and female loons

Year	# Territories monitored	# Territorial pairs	# Nesting pairs	# Chicks hatched	# Chicks fledged	# Chicks fledged/ territorial pair
1996	89	81	58	46	31	0.38
1997	115	103	68	65	47	0.46
1998	129	124	96	51	35	0.28
1999	134	128	83	71	50	0.39
2000	183	181	127	116	66	0.36
2001	189	179	124	102	61	0.34
2002	182	163	110	81	52	0.32
2003	173	160	105	101	62	0.39
2004	169	155	104	104	68	0.44
2005	166	148	102	84	61	0.41
Total	1529	1422	977	821	533	0.37

Table 1 Number of territorial pairs and reproductive success of common loons in the Maine and New Hampshire study area



Fig. 6 Mean changes (\pm SE) in overall reproductive success (chicks fledged per territorial pair, CF/TP) in relation to Hg levels measured as female loon unit (FLU) equivalents (ug/g, ww)

conditions that favor methylation of Hg, which can lead to biological Hg hotspots (Driscoll et al. 2007, Evers et al. 2007). Below, we describe our findings for each of our endpoints, including the combined effects and implications for the sustainability of breeding populations. We also identify geographic areas that are most likely to contain population sinks.

Behavioral impacts

Time spent on the nest is a key behavior for detecting abnormalities related to MeHg body burdens. In our study, we quantified adult behavior through TABs during three distinct breeding periods: pre-nesting, nesting and postnesting. Others have reported relationships between Hg and decreased activity levels (or increased lethargy), motivation to hunt, and thermoregulation (Heinz 1996; Thompson 1996; Bouton et al. 1999).

We found that Hg can negatively impact normal adult loon behavioral activities and grouped these activities into two broad categories based on other studies: activity level and incubation time. Higher blood Hg concentrations are correlated with increased lethargy in adults (Fig. 3). Highenergy behavioral events, such as time adults spent foraging for chicks and themselves, declined as blood Hg levels increased. The reduction in time spent foraging could contribute to lowered chick survival.

Average time spent on the nest declined from the normal coverage of 99% to 86% of the time as Hg exposure increased from ≤ 1.0 ug/g to ≥ 3.0 ug/g. Incubation is one of the few behaviors for which we recorded notable impacts from Hg during field observations. Generally, a breeding pair equally shares incubation (Evers 1994; Mager 1995; Paruk 2000). In several cases, on loon territories where blood Hg levels ranged from 4.1 ug/g to 6.7 ug/g in females and 4.4 ug/g to 7.3 ug/g in males, breeding individuals exhibited aberrant incubation behavior.

The loon territory at Stratton Island on Flagstaff Lake is a representative example. This territory was monitored dawn to dusk in June of 1999. Observers documented the routine of the non-incubating male approaching the incubating female using normal, subtle behaviors including soft mew calls, peering, and maintaining a low body profile. The female responded normally by moving off her eggs and swimming underwater, and surfacing 15 30 m from the nest. She regularly moved out into the territory to feed during the next 4 6 h. However, instead of incubating the eggs, the male remained on the water and patrolled the nest site. After the eggs were exposed to ambient temperatures and the elements for 4 6 h, the female returned, switched nest duties with the male, and moved to an incubation posture. This nest was abandoned after five days.

Unattended eggs have a higher probability of being chilled/overheated or predated, which likely results in a higher incidence of nest failures. Such behavior and related nest failures were observed on other territories indicating that overt abnormal behavior can be quantified at elevated Hg levels.

Physiological impacts

Several biomarkers were evaluated to determine potential physiological effects of the blood Hg concentrations in this study. Tests using these biomarkers varied from no adverse effects detected in immunosuppression (i.e., white blood cell counts) (Haefele et al. 2005) and genetic damage (i.e., DNA fragmentation) (Emery 2007) endpoints to significant yet coarse adverse effects in hormonal changes (i.e., corticosterone levels) (Franceschini 2007). The strongest relationship with Hg was the test of asymmetry between remige weights.

Using flight feathers, we measured the relationship between 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 (1995) and Polak and Trivers (1994) suggested FA to be a sensitive measure of long-term body condition, and Yablokov (1986) and Moller and Swaddle (1997) considered FA as a sentinel for subtle environmental perturbations prior to visible effects in population viability. Some studies have linked heavy metal pollution to increased asymmetry in primaries (Eeva et al. 2003).

It appears that the loon's remige weights are a sensitive indicator of FA and the relationship of FA with high Hg risk breeding loon populations makes this biomarker important for monitoring age-confounded impacts from Hg. Although other stressors may disrupt developmental homeostasis, and genetic diversity may predispose some populations to having greater FA than others (especially in the loon, e.g., Dhar et al. 1997), we found that FA is a valid endpoint of physiological stress from Hg. Symmetricallymeasured remige weights with average differences of 6.7% found on individual loons with feather Hg levels \geq 40 ug/g could have substantial impacts on individual fitness. For example, flight studies in wind tunnels found a wing area with a difference of 5% to result in 20% more energy expenditure to fly in the European starling (*Sturnus vulgaris*) (Swaddle 1997). Further, lower wing asymmetry in migrating cliff swallows (*Petrochelidon pyrrhonota*) corresponded with significantly greater survivorship during an extreme climatic disturbance that reduced a local population by over 50% (Brown and Brown 1998).

Survival impacts

Although birds have natural defense mechanisms for depurating (e.g., feathers), demethylating (e.g., liver and kidney), and sequestering (e.g., egg) Hg (Evers et al. 2005), 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 loons 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 reflects the available body burden of MeHg in an individual loon.

In general, feather Hg increased in known individuals by an average of 8.4% per year, which is similar to earlier findings by Evers et al. (1998). However, feather Hg levels exceeding 30.0 ug/g tended to have a greater average annual increase of 10%. The increase of feather Hg levels with age indicates greater risk of Hg impacts to older individuals. Scenarios where birds are at lower risk indicate no evidence of bioaccumulation in body burdens, such as in known-aged great skuas (*Catharacta skua*) (Thompson et al. 1991).

Although Mitro et al. (2007) did not significantly correlate Hg body burdens to apparent adult survivorship, their elasticity analysis found that an exceedingly large spatiotemporal sample size is required to detect a significant impact of Hg on small differences in loon survival (likely the case because of the loon's relatively long lifespan that may exceed 30 years). Because there can be substantial annual increases in feather Hg levels, understanding how rising Hg body burdens in high risk adults could impact their reproductive success will require further detailed study on known individuals.

Reproductive impacts

Our emphasis on the analysis of the CF/TP measure is based on the high confidence of data (i.e., nests can fail prior to detection and hatched chicks can die before confirmation) and the biological significance of an outcome of a fledged chick. By standardizing loon Hg levels in blood and eggs, we used a FLU to establish a relationship between CF/TP in correspondence with Hg concentrations. Such an analysis demonstrates a significant decline in reproductive success from low to higher Hg levels that is strongly predictive ($r^2 = 0.86$) (Fig. 6).

Our dataset indicates a break in CF/TP categories at adult blood Hg levels of 3.0 3.5 ug/g that is significantly lower than the control group (<1.0 ug/g) (t = 2.38, df = 19, p = 0.02). In our study population, adult loons with blood Hg levels ≥ 3.0 ug/g produce 41% fewer fledged young than breeding loons with blood Hg levels ≤ 1.0 ug/g. We predict total reproductive failure when adult blood Hg levels reach 16.5 ug/g. Burgess and Meyer (this issue) had similar findings based on a linear upper limit of fledging success; they documented a loss of approximately 40% of fledged young when adult blood Hg levels reached 3.45 ug/g.

Integrating endpoint measurements

The separate endpoints we analyzed provide compelling evidence of negative impacts on neurological, physiological, and reproductive abilities of breeding loons due to high Hg exposure. They also provide insight into why there is ultimately lowered reproductive success in loons as Hg concentrations increase. We speculate that flight feather asymmetry could decrease migratory performance for a species where wing-loading is restrictive (Mager et al. 2007). Extra energy demands stemming from migratory flights could impact a loon's ability to maintain a breeding territory against intruding adults that regularly evaluate individual fitness; prospecting by both pre-breeders and displaced breeders is known to use cues of fitness at an established loon's breeding territory (Piper et al. 2006). Earlier-than-normal and more frequent loss of a breeding territory would therefore occur for individuals with elevated body burdens of Hg.

The mechanism through which developmental instabilities manifest into behavioral aberrations is relatively unknown; however, the neurotoxic nature of MeHg appears to impact adult loons by reducing time spent incubating, as well as causing a lethargic response during a high-energy time period of feeding chicks. Breeding adults that reside in Hg sensitive areas are also prone to annual increases of Hg body burdens that result from an inability to sufficiently depurate and demethylate dietary uptake of Hg. Prey item Hg levels that are correlated with reductions of 40% and 41% in fledging success are 0.16 ug/g (ww) (Evers et al. 2004) and 0.17 ug/g (ww), respectively (Burgess and Meyer, this issue). Prey fish behavior may also succumb to elevated Hg levels; studies have found cyprinnids with 0.52 ug Hg/g (whole body, ww) to school erratically (Webber and Haines 2003), which could lead to lethargic loons disproportionally selecting fish that exhibit inhibited predator-avoidance behaviors.

Based on our endpoints, areas of high Hg exposure could result in age-related increases in Hg concentrations that could reduce an individual's lifetime reproductive success (LRS) and alter the population's age-structure toward younger individuals. Generally, breeding performance is lowered in seabirds when older individuals are replaced by younger ones (Sydeman et al. 1991), mate changes occur (Wooller et al. 1988), and body condition is compromised (Chastel et al. 1995). High quality parents generally account for the majority of young recruited in a local population (Newton 1989); in red-billed gulls (Larus novaehollandiae scopulinus) only 8% of the eggs laid resulted in recruitments that bred (Mills 1989). A detailed demographic study based on known individual common loons is needed to document the relationship between Hg and LRS. Such an effort should examine if high quality parents tend to choose territories that are in habitats sensitive to MeHg production and accessibility.

Conclusions

In our Maine New Hampshire study, Hg appears to be a primary anthropogenic stressor reducing reproductive success to significantly under 0.48 CF/TP. Here, 16% of the adult population exceeds tissue thresholds that have significant adverse effects (Table 2). Based on a population matrix model, loon breeding populations producing fewer than 0.48 CF/TP are characterized as population sinks using model parameters of an annual adult survivorship of 92%, subadult survival of 41%, and average first year breeding age of 6 years (Mitro et al. 2007; Evers 2007). Influence from extrinsic factors such as weather, predation, habitat variability, and human disturbance are undoubtedly present and need to be factored into a comprehensive risk assessment (Nacci et al. 2005); however, because the average CF/TP did not significantly differ among years, the contribution of intrinsic and extrinsic factors appears to be constant and therefore predictive.

While there are areas where Hg is negatively impacting loon population viability in Maine and New Hampshire, lessstudied areas in the loon's extensive core breeding range in Ontario and Quebec need further study. These and other areas in Canada are generally more sensitive to sulfate deposition and subsequent acidification of lakes than New England (Tan 1989) and therefore are even more sensitive to the deposition of atmospheric Hg. The lowering of the pH in lake systems is one factor that is associated with enhanced MeHg concentrations in surface water and elevated fish Hg levels (Driscoll et al. 2001, 2007). Sulfate deposition further enhances methylation by supporting an increase in sulfurreducing bacteria that drive the methylation process (Jeremiason et al. 2006). There are indications that biological Hg hotspots exist in Canada where loon reproduction may be **Table 2** Summary ofthresholds for adverse effects inloon tissues and prey items

Matrix	Adverse effect threshold	Based on	Reference
Adult blood	3.0 ug/g (ww)	Reproductive failure	This paper
Adult feather	40.0 ug/g (fw)	Flight feather asymmetry	This paper
Egg	1.3 ug/g (ww)	Reduced volume	Evers et al. 2003
Prey fish	0.16 ug/g (ww)	Relationship with adult blood Hg levels	Evers et al. 2004

negatively affected (Evers et al. 2007), including the La-Maurice area in Quebec (Champoux et al. 2006), southern Ontario (Scheuhammer et al. 1998), and southern Nova Scotia (Burgess et al. 2005).

Spatial gradients and temporal trends in loon Hg burdens can be used to identify areas at risk for ecological and human health impacts in North America. The creation of an international Hg monitoring program that includes avian piscivores, such as the common loon, would provide a standardized approach to identify biological Hg hotspots and track changes in Hg deposition. The methods for such a project in the United States are now established (Mason et al. 2005, Harris et al. 2007). Loons are identified as one of the higher ranked avian piscivorous bioindicators (Wolfe et al. 2007) and their Hg body burdens are well-established as providing an approach for measuring both biological Hg hotspots and tracking the impacts of temporal changes in Hg deposition (Evers et al. 2007). Expanding this approach to other northern hemisphere countries would greatly contribute to international concerns for global action (Selin 2005). Further monitoring will provide the long-term datasets that are needed for conservation planning, thereby facilitating national and even global decisions for regulating Hg emissions. Such attention will reduce the magnitude and extent of population sinks created by anthropogenic Hg sources for loons as well as other wildlife.

Acknowledgments This study was and continues to be an extensive effort of BRI's International Center for Loon Conservation. Numer ous individuals, organizations and agencies assisted with this study. The Maine Department of Environmental Protection, FPL Energy Maine Hydro, the U.S. Fish and Wildlife Service, and Rawson Wood were instrumental for funding major aspects of this study. Robert Poppenga of the University of Pennsylvania supervised lab analysis for mercury in blood and feathers, and Robert Taylor of Texas A&M's Trace Element Research Laboratory analyzed egg mercury levels. EarthWatch Institute volunteers assisted with the collection of behavioral observations. 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 and the many field staff and volunteers who contributed their expertise and enthusiasm to this study.

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