

Bi-phasic trends in mercury concentrations in blood of Wisconsin common loons during 1992–2010

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Abstract We assessed the ecological risk of mercury (Hg) in aquatic systems by monitoring common loon (*Gavia immer*) population dynamics and blood Hg concentrations. We report temporal trends in blood Hg concentrations based on 334 samples collected from adults recaptured in subsequent years (resampled 2–9 times) and from 421 blood samples of chicks collected at lakes resampled 2–8 times 1992–2010. Temporal trends were identified with generalized additive mixed effects models and mixed effects models

to account for the potential lack of independence among observations from the same loon or same lake. Trend analyses indicated that Hg concentrations in the blood of Wisconsin loons declined over the period 1992–2000, and increased during 2002–2010, but not to the level observed in the early 1990s. The best fitting linear mixed effects model included separate trends for the two time periods. The estimated trend in Hg concentration among the adult loon population during 1992–2000 was -2.6% per year, and the estimated trend during 2002–2010 was $+1.8\%$ per year; chick blood Hg concentrations decreased -6.5% per year during 1992–2000, but increased 1.8% per year during 2002–2010. This bi-phasic pattern is similar to trends observed for concentrations of methylmercury and SO_4 in lake water of an intensely studied seepage lake (Little Rock Lake, Vilas County) within our study area. A cause-effect relationship between these independent trends is hypothesized.

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Introduction

Two decades of research in Wisconsin has probed the relationship between mercury (Hg) deposition, lake chemistry, and the availability of Hg in the aquatic food chain (Bloom et al. 1991; Hurley et al. 1991; Watras et al. 1994; Watras et al. 2005). Regional atmospheric Hg deposition, as well as fish and wildlife Hg concentrations, are elevated in Wisconsin and the western Great Lakes region, particularly in low alkalinity lakes (Cope et al. 1990; Evers et al. 1998; Meyer et al. 1995, 1998; Wiener et al. 2003). Mercury concentrations in Wisconsin aquatic

ecosystems began declining in the 1980s (Fevold et al. 2003; Hrabik and Watras 2002; Rasmussen et al. 2007; Watras et al. 2000). Similar patterns of decline were reported in Minnesota (Monson 2009), and other regions of the Great Lakes (Bhavsar et al. 2010); however, rates of decline have varied across region, with lake type, and with land use (Chen and Folt 2005; Rasmussen et al. 2007; Vanarsdale et al. 2005; Monson 2009; Watras 2009). Several factors are associated with trophic transfer of methylmercury (meHg) in aquatic systems and likely account for this variability. The in-lake Hg methylation cycle depends on factors such as lake-pH, SO₄, dissolved organic carbon (DOC), P, microbial activity, water budget, precipitation, and Hg^{II} deposition from external sources (Watras and Morrison 2008), and in-lake meHg production can be up to seven times greater than methylmercury loading from adjacent wetlands. Some of these factors interact to influence the Hg methylation process independent of ongoing Hg deposition or historical loading of Hg within a lake's catchment area or watershed (Watras and Morrison 2008). Top predator wildlife and fish are the ultimate receptors of meHg trophic transfer in aquatic systems, thus most exposed and provide the best index of meHg toxicity risk. Temporal tracking of wildlife Hg concentrations may provide an index of risk associated with changing atmospheric Hg deposition patterns as they integrate the multiple factors associated with bioaccumulation of meHg.

The common loon (*Gavia immer*) is a high trophic level, long-lived, obligate piscivore. Loons are sensitive to the toxic effects of Hg (Barr 1986; Scheuhammer et al. 1998; Nocera and Taylor 1998) and have elevated risk of meHg exposure relative to other wildlife species on inland (non-marine) North American aquatic systems. Loons nesting on low-pH lakes in northern Wisconsin have elevated Hg levels in their blood and eggs and exhibit reduced reproductive performance (Meyer et al. 1995, 1998). Studies in New England, Ontario, and the Canadian Maritimes have found similar patterns between Hg exposure and loon performance (Barr 1986; Burgess and Meyer 2008; Nocera and Taylor 1998; Scheuhammer et al. 1998, 2007). Field and laboratory dosing experiments with meHg have documented changes in common loon hatching rates, immune function, behavior, oxidative stress, and neural histology (Kenow et al. 2007a, b, 2008, 2010, 2011) associated with ecologically relevant doses.

The Wisconsin Wildlife Biosentinel Program assesses the ecological risk of Hg in aquatic systems by conducting long-term monitoring of Hg concentrations in blood of common loons. Previously, Fevold et al. (2003) reported a decline in northern Wisconsin loon blood Hg concentrations during the period 1992–2000. Hrabik and Watras (2002) describe a similar decline in Hg in yellow perch

(*Perca flavescens*), a primary loon prey item (Merrill et al. 2005), during 1988–2000 in Little Rock Lake, a seepage lake within our loon sampling region (Fig. 1). The objective of this study is to extend the period of common loon Hg exposure assessment through 2010.

Methods

Study lakes were located in a 4-county region (Vilas, Oneida, Forest, and Iron counties) of northern Wisconsin centered at approximately 46°N and 89°30'E (Fig. 1). The study lakes were oligotrophic to mesotrophic ecosystems with a wide range of pH (4.9–8.5), acid neutralizing capacity (–9–1513 µeq/l), apparent color (3–125 PCU), and conductivity (10–160 µS/cm @ 25°C) (Wisconsin DNR, unpublished data). Lake pH was quantified with previously described methods (Meyer et al. 1998). Loon sampling during 1992–2000 (136 captures of 71 individual loons) included several low-pH lakes, where meHg exposure is greatest, to quantify the range of meHg exposure in Wisconsin loons (Meyer et al. 1998). Loons were sampled during 2002–2010 (198 captures of 85 individual loons) on a randomly selected subset of these lakes, chosen to be representative of all lakes in the region, with the goal of developing a loon population model (Gear et al. 2009).

Loon capture and tissue sampling

Loon adults and chicks were captured during the summers of 1992–2010 with a night-lighting technique (Evers 1993). Loon are captured by a crew of 3 in a small boat equipped with a 6HP outboard motor, landing net, spotlights, and taped loon calls. Adults and chicks are placed in holding boxes and transported to shore for processing. A total of 1072 loon blood samples were collected during 2002–2010 from adult loons and chicks on 178 lakes, and total Hg concentrations were assessed. Results from adults that were resampled (e.g., captured in 2 or more years) or lakes where chicks were sampled more than once (e.g., chicks sampled in 2 or more years) were pooled with those from the 1992–2000 analyses, and temporal trend analyses were conducted. Observations were excluded if data on lake pH were not available (9 observations from 4 loons) or if loons moved to a different lake (5 observations on 5 loons). While loon chick age was not known precisely when chicks were sampled (lakes were visited 4–6 times prior to banding to ascertain hatching status), we estimated chick age based on size and plumage, and timed capture to coincide with loon chick age of 5–7 weeks as they were sufficiently large to band but still vulnerable to our capture technique. When sibling chicks were sampled, results were averaged. Blood was drawn from adults and chicks from the distal portion of the femoral vein with a 10-cc plastic syringe with a 21-gauge

Fig. 1 The geographic context of the Wisconsin Common Loon Sample Area within the western Great Lakes Region. Little Rock Lake (46°00'N, 89°42'W) is located near the center of the study area. State abbreviations are IA (Iowa), IL (Illinois), MI (Michigan), MN (Minnesota), and WI (Wisconsin)



hypodermic needle. Chick blood was collected with the same technique, but with a 25- or 27-gauge needle and 3- or 5-cc syringe. Whole blood collected during 1992–2006 was transferred to heparinized Vacutainers[®] (green top), preserved with a 1:20 ratio of 10% formalin to whole blood, and kept frozen until submitted for mercury analysis. Samples collected during 2007–2010 were transferred to plastic cryovials and stored frozen until analysis. Following blood collection, birds were banded with a unique combination of colored plastic leg-bands and a numbered US Fish and Wildlife Service #8 aluminum band. Birds were then weighed with a 5- or 10-kg Pesola spring-scale and released. Some loon adults were captured 2–5 times during 1992–2010. Blood samples from each capture occasion were analyzed for total Hg.

Mercury analysis

The concentrations of total Hg in 1992–2005 loon blood samples were determined by cold-vapor atomic absorption (CVAA) spectrophotometry (detection limit = 0.01 µg Hg/ml; En Chem, Inc., Madison, WI, USA) following USEPA

SW-846 Method 7471A. Reported blood Hg concentrations were adjusted (multiplied by 1.05) to account for formalin added to samples collected 1992–2005. Method blanks and standard reference material (certified dog-fish liver) were processed and analyzed concurrently with loon tissue samples. Blood samples collected during 2006–2010 were analyzed by CVAA at the Wisconsin State Laboratory of Hygiene (WSLH) in Madison. All laboratories (En Chem, Inc. and WSLH) used similar standardized sample preparation and acid digestion procedures (En Chem, Inc., USEPA SW-846 Method 7471A; WSLH, ESS INO Method 540.4, Sullivan and Delfino 1982), and CVAA spectrophotometry protocol (En Chem, Inc., USEPA SW-846 Method 7471A; WSLH ESS INO IOP 540, Sullivan and Delfino 1982). In addition, each set of ≤ 20 samples included a method analytical blank and a matrix control spike that were digested and analyzed with the samples. A certified biological standard reference material (SRM) Dogfish DORM-1, lobster, or tuna) was also digested and analyzed with each batch of samples. Control spikes were certified SRM (tuna fish, catfish fillet, or oyster tissue) spiked with a known quantity of mercury prior to digestion. One tissue sample

from each batch was also chosen for a matrix spike and spike duplicate analysis. All data used in the trend analysis met the certified laboratory's QA/QC standards.

We acknowledge that there is potential for inter-laboratory error when comparing tissue Hg concentrations that have been obtained from multiple analytical laboratories. However, potential inter-laboratory error is an inherent problem associated with most long-term contaminant studies. In this study, both labs are accredited by NELAP (National Environmental Laboratory Accreditation Program) and the Wisconsin Department of Natural Resources (NR 149) for environmental testing of mercury. As such both labs have on-site inspections to review procedures and test Hg PT samples for water and solid samples.

Methods of analysis of trends in loon blood mercury

We used log-transformed Hg concentrations ($\log_{10}[\text{Hg}]$) as the response variable for analyses because the variance of residuals was more homogeneous on this scale. Environmental contaminants such as Hg are often lognormally distributed because of the dilution processes involved in their generation (Ott 1995). Linear trends in log concentration correspond to exponential trends in concentration and can be expressed as a constant percent change per year.

Adult loons in this study were recaptured up to 5 times (i.e., in 5 different years), and as many as 9 loon chicks were sampled on some lakes. Repeated observations of the same adult loons are clearly not independent. Similarly, data from chicks on the same lake cannot be assumed to be independent because the chicks may have the same parents, and because lake characteristics that influence chick Hg concentration remain constant. We analyzed both the adult and chick data with mixed effects models to account for the potential lack of independence among observations from the same loon (for adults) or same lake (for chicks) (Littell et al. 1996; Zuur et al. 2009). Fixed effects included lake pH, year sampled (measured as the number of years since 1991 and treated as a continuous variable), and the interaction between pH and year. Because sampling occurred during two 9-year periods (1992–2000 and 2002–2010) separated by a 1-year interval, we fit some models with separate fixed effects for year in each time period, allowing the trend to differ between the periods, as well as models with one trend for the entire period of 1992–2010. Models with separate trends for each time period were parameterized so that the term that is normally the intercept is the estimated $\log_{10}[\text{Hg}]$ concentration in 2001, at the point where the two trend lines intersect (Draper and Smith 1981), adjusted by the pH of each lake. For analyses of adult loon data, we also included fixed effects for year of first capture and for years since first capture to investigate

the temporal trend among loons and the rate of bioaccumulation of Hg within loons.

Random effects associated with adult loons or with lakes (for models of chick blood Hg) allowed estimates for individual loons or lakes to deviate from the population model determined by the fixed effects. We included random effects for the intercept and slope of the $\log_{10}[\text{Hg}]$ —year relationship. We used Akaike Information Criterion (AIC) to select among the linear mixed effects models; the model with the minimum value of AIC provides the best balance between bias and precision (Burnham and Anderson 2002). Models within 2 AIC units of the smallest AIC value were examined in greater detail, as recommended by Burnham and Anderson (2002). Model comparisons with AIC were based on maximum likelihood (ML) estimation, whereas final parameter estimates were obtained with restricted maximum likelihood (REML) (Verbeke and Molenberghs 2000). We report *P*-values based on Wald tests with degrees of freedom computed by the containment method, which gave fewer degrees of freedom, and thus more conservative tests, than other methods. Computation of denominator degrees of freedom associated with tests of fixed effects in mixed effects models is difficult and there is no general agreement on which method is best (Fitzmaurice et al. 2004). Computing was done with the SAS PROC MIXED procedure (Littell et al. 1996).

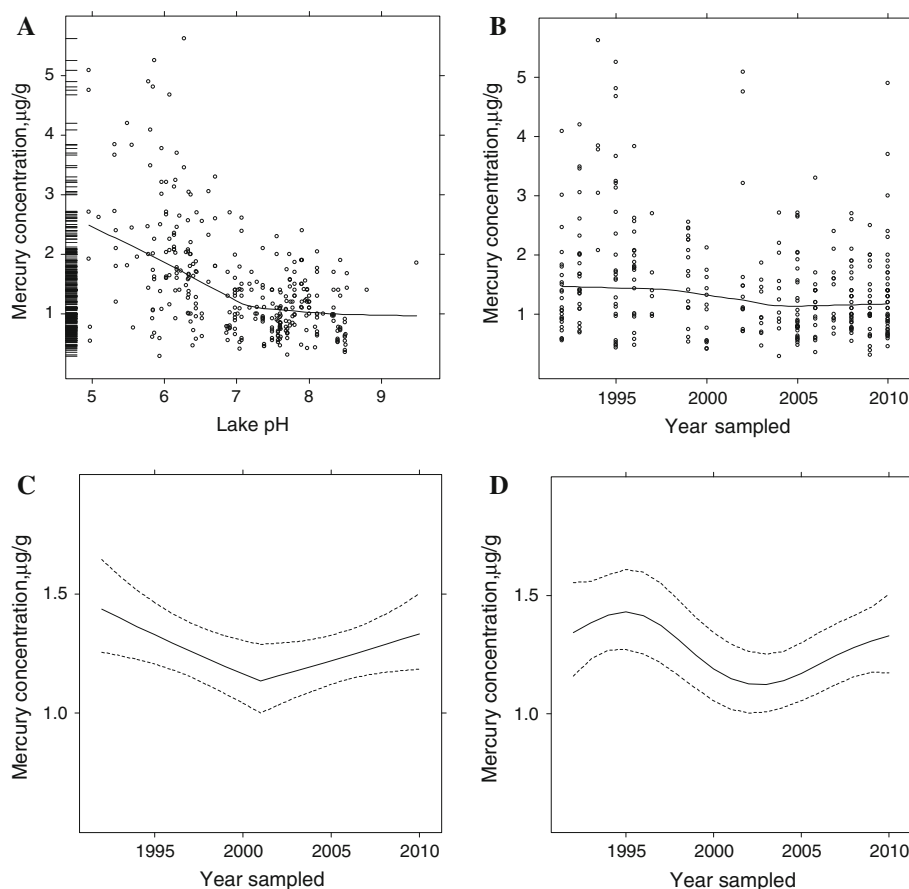
Time trends are not necessarily linear, and changes in time trends need not correspond to periods of sampling; for this reason we also fit generalized additive mixed effects models (GAMMs) that included a linear effect of lake pH on $\log_{10}[\text{Hg}]$, but a smooth function based on regression splines to model the effect of time (Wood 2006; Zuur et al. 2009). Interpretation of time effects from the GAMM models was carried out through graphical presentation of model estimates because of difficulties in interpreting coefficients for smooth regression splines (Venables and Dichmont 2004). We fit GAMM models with the R package *mgcv* with smooth terms represented by thin plate splines (Wood 2006).

Results

Adult loon blood Hg concentrations

Trend analyses for adult loons were based on 334 blood Hg concentrations from 156 loons on 120 lakes in northern Wisconsin. The distribution was positively skewed, with a maximum adult loon blood Hg concentration of 5.62 $\mu\text{g/g}$, a median of 1.23, and 75th percentile of 1.80 (see Fig. 2a). Blood Hg appeared to decline as lake pH increased (Fig. 2a). Observations were distributed throughout the period 1992–2010 (Fig. 2b).

Fig. 2 Wisconsin adult loon **a** blood mercury concentration ($\mu\text{g/g}$) versus lake pH (*short horizontal bars* along the y-axis show the distribution of the mercury concentrations), **b** blood mercury concentration versus year sampled, **c** estimates (*solid line*) and 95% confidence interval (*dashed line*) for blood mercury concentration from linear mixed effects model 1 (see Table 1 for list of models) for a lake of pH 7, **d** estimates (*solid line*) and 95% confidence interval (*dashed line*) for blood mercury from the GMM model for a lake of pH 7. *Solid lines* in **a** and **b** show the central tendency of mercury concentration with respect to lake pH (**a**) and year (**b**) based on a loess smooth for one predictor variable



Previous analyses of trends in adult loon blood Hg suggested a difference between the temporal trend among loons, based on first capture, and the rate of bioaccumulation within loons, based on repeated measurements of recaptured loons (Fevold et al. 2003). With data extending for an additional decade, the models with different among-loon and within-loon trends (Table 1, Models 6 and 8) did not fit as well as other models. The linear mixed effects model with lowest AIC (Table 1) included separate trends for the two periods, 1992–2000 and 2002–2010 (Fig. 2c). The estimated trend in Hg concentration for adult loons during 1992–2000 was -2.6% per year ($(10^{-0.0114} - 1) \times 100$), with a 95% confidence interval extending from -4.6% to -0.5% , and the estimated trend during 2002–2010 was 1.8% per year ($(10^{0.0077} - 1) \times 100$), with a 95% CI of -0.2% to 3.9% . These are similar to the trend estimates obtained by dividing the data into two subsets by time period and carrying out separate analyses of each. Based on model 1 (Online Resource 1—Tables 1 and 2), the effect of lake pH on loon blood Hg concentration was to decrease it by -25.5% (95% CI -30.8 to -19.6%) for each one-unit increase in pH. For a lake of pH 7, for example, the model indicated that the population trend lines intersect in 2001 at a $\log_{10}[\text{Hg}]$ of $0.948 - 0.127 \times 7 = 0.059$, corresponding to a Hg concentration of $1.15 \mu\text{g/g}$. This model included random effects for loons associated with intercepts, but not slopes, of the

$\log_{10}[\text{Hg}]$ —year relationship, so that estimates for individual loons differed from the population intercept for a lake of a given pH, but did not differ from the population trend for lakes of that pH. A similar model that included random effects for slopes (Table 1, model 3) fit less well and yielded almost identical estimates for the population trends with respect to pH and time.

A GMM model qualitatively similar to model 1 also suggested an initially decreasing trend in adult loon blood Hg concentration, followed by an increase later in the sampling period (Fig. 2d). The lowest estimated Hg concentration from this model occurred in 2003, with slowly increasing concentrations in following years. The effect of lake pH on $\log_{10}[\text{Hg}]$ as estimated by the GMM model was similar to that of the linear mixed effect model (-0.123 vs. -0.128 ; see Online Resource 1—Table 1).

The linear mixed effects model with the second lowest AIC included an interaction between pH and year, suggesting that the rate of change in Hg concentration over time depends on lake pH, with more rapid decreases over time in more acidic lakes (parameter estimates in Online Resource 1—Table 2). Parameter estimates from model 2 imply, for instance, that adult loons on lakes of pH 6 have experienced a decline in Hg of -1.7% per year, while those on lakes of pH 7 have experienced a decline of -0.5% per year, and on lakes of pH 8, loon blood Hg has increased by

Table 1 Linear mixed effects models fit by maximum likelihood (ML) to adult loon data, listed in order with the best-fitting model first

Model	Fixed effects	Random effects	Δ AIC
1	pH + yr1 + yr2	Intercept	0.0
2	pH \times year	Intercept	1.0
3	pH + yr1 + yr2	Intercept + slopes	1.9
4	pH	Intercept	2.3
5	pH \times year	Intercept + slope	3.0
6	pH + year	Intercept	3.8
7	pH \times (start + follow)	Intercept	5.0
8	pH + year	Intercept + slope	5.8
9	pH + start + follow	Intercept	5.8

The difference in Akaike's Information Criterion (AIC) from the model with minimum AIC is listed as Δ AIC. Models with fixed effects denoted as "pH \times year" include effects of pH, year, and their interaction; fixed effects denoted as "pH + year" include effects of pH and year only. Fixed effects listed as "yr1" and "yr2" are year effects in time period 1 (1992–2000) and period 2 (2002–2010), respectively. Models with random effects for the intercept allow individual loons to deviate from the population intercept of the trend relationship. Models with the additional random effect for slope allow individual loons to deviate from the population slope of the trend relationship

0.7% per year. As with model 1 (Table 1), this model included random effects for loons associated with intercepts, but not slopes, of the $\log_{10}[\text{Hg}]$ –year relationship.

Loon chick blood Hg concentrations

Blood Hg concentrations were obtained from 421 chicks on 118 lakes. The mean chick mass (s.d.) at banding over the sampling period was 1999 g (405), while the mean for the period 1992–2000 was 1925 g (393) and that for the period 2002–2010 was 2029 g (407). Lake pH was not available for 7 of the lakes with a total of 21 chicks sampled, so the statistical analyses reported here were based on 400 blood Hg concentrations from 111 lakes. The distribution of chick blood Hg concentrations was strongly positively skewed, with a maximum of 0.904 and a median of 0.10; 75% of all observations were below 0.1815 (Fig. 3a). Most of the high blood Hg concentrations were from lakes with low pH (Fig. 3a). Samples were obtained throughout the period of 1992–2010 (Fig. 3b).

Trend analyses indicated that Hg concentrations in the blood of loon chicks declined over the period 1992–2000, but increased during 2002–2010. The best fitting linear mixed effects model (see Online Resource 1—Tables 3 and 4) included separate trends for the two time periods, suggesting that chick blood Hg concentrations decreased by -6.5% per year (95% CI -9.0% to -3.9%) during 1992–2000, but increased by 1.8% per year (95% CI -0.05% to 3.7%) during 2002–2010 (Fig. 3c). Similar estimates of trend can be

obtained by splitting the data into two subsets based on time period and carrying out separate analyses of each. As with the similar model for adult loons, this model was parameterized so that the $\log_{10}[\text{Hg}]$ at the intersection of the two trend lines in 2001 was estimated as a coefficient, with an adjustment for lake pH. Thus, for a lake of pH 7, the population trend lines were estimated to intersect at a $\log_{10}[\text{Hg}]$ of $0.708 - 0.245 \times 7 = -1.007$, corresponding to a Hg concentration of 0.10. The effect of lake pH in this model was to decrease chick blood Hg concentration by -43.1% per unit increase in pH (95% CI -51.1% to -36.7%). The two models with lowest AIC had the same fixed effects terms, but differed in random effects (see Online Resource 1—Table 3). Model 1 included random effects for both the intercept and slopes of the $\log_{10}[\text{Hg}]$ –year relationship, indicating that for individual lakes, both the estimated mean Hg and the rate of change in Hg varied with respect to the population model given by the fixed effects. Model 2 resulted in similar estimates of fixed effects, but did not fit as well because of the simpler model for random effects.

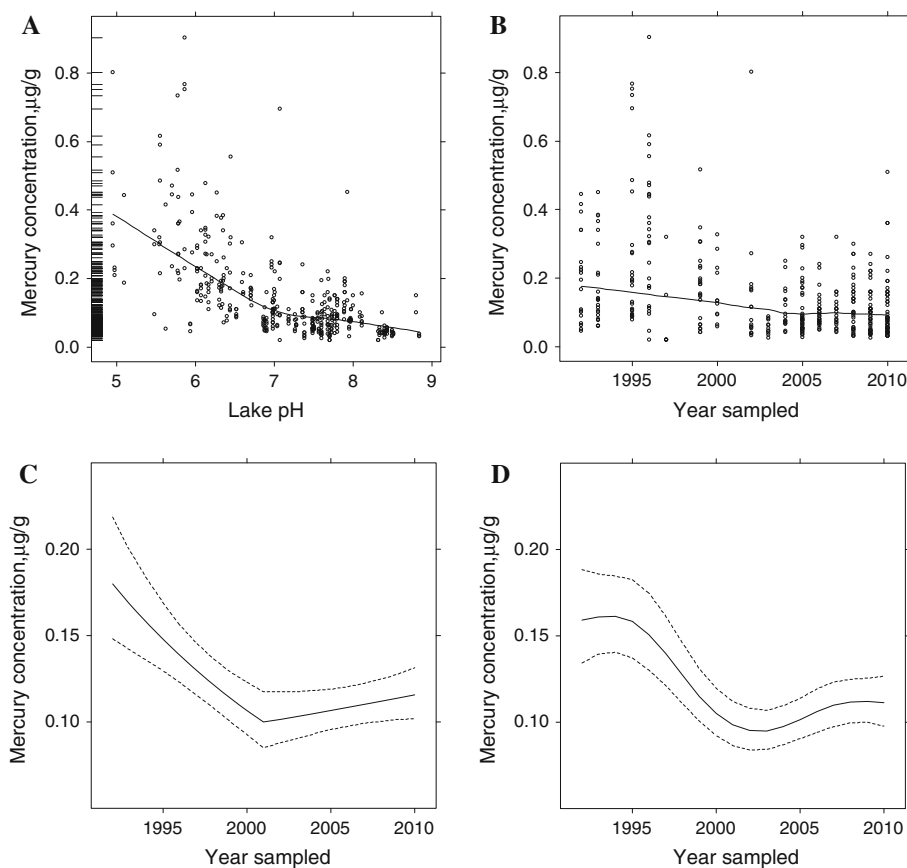
The GAMM model for chick blood Hg suggested a similar pattern of change over time, with decreasing Hg concentrations until 2003 and increasing concentrations thereafter (Fig. 3d). As with the adult loons, the effect of lake pH estimated by the GAMM model (-0.236) was similar to that from the linear mixed effects model (-0.245).

Discussion

A significant change in the trend of Wisconsin loon blood Hg concentration occurred in the early 2000 s. At least three factors could explain the trend reversal: (1) sampling or analytical bias, (2) increased mercury deposition, and (3) increased meHg bioaccumulation. Our study, in conjunction with other studies of trends in Hg in fish and of meHg in lakes, indicates that the third factor may have primary importance.

Loon sampling in Wisconsin 1992–2000 included several low-pH lakes, where meHg exposure is greatest, to quantify the range of Hg exposure in Wisconsin loons (Meyer et al. 1998). Loons sampled during 2002–2010 were found on a randomly selected subset of these lakes, chosen to be representative of all lakes in the region, with the goal of developing a loon population model (Gear et al. 2009). As a consequence, the mean lake pH of 6.7 in 1992–2000 was lower than that of 7.4 in 2002–2010. Because of the strong relationship between lake pH and meHg exposure, it was essential to account for the effects of lake pH in our trend analyses of Hg in loon blood. Those analyses clearly indicated the importance of lake pH in determining loon blood Hg levels, with adult blood Hg levels decreasing by 25.5% and chick blood Hg levels

Fig. 3 Wisconsin loon chick **a** blood mercury concentration ($\mu\text{g/g}$) versus lake pH (*short horizontal bars* along the *y*-axis show the distribution of the mercury concentrations), **b** blood mercury concentration versus year sampled, **c** estimates (*solid line*) and 95% confidence interval (*dashed line*) for blood mercury concentration from linear mixed effects model 1 (see Table 4 for list of models) for a lake of pH 7, **d** estimates (*solid line*) and 95% confidence interval (*dashed line*) for blood mercury from the GAMM model for a lake of pH 7. *Solid lines* in **a** and **b** show the central tendency of mercury concentration with respect to lake pH (**a**) and year (**b**) based on a loess smooth for one predictor variable



decreasing by 43.1% for each unit increase in pH. There was some evidence for an effect of lake pH on temporal trends for adult loon blood Hg, but less so for chicks. It is possible that a stronger biphasic temporal trend in loon blood Hg levels is accompanied by a weaker effect of lake pH on trends, with greater declines in more acidic lakes.

Differences in loon mass or age at the time of sampling could be another methodological factor biasing a study of this kind. Kenow et al. (2003) found that loon chick blood Hg varied with chick age when chicks were fed a diet containing a constant concentration of meHg in a controlled laboratory study. A subsequent field study within the same study area indicated that blood Hg concentration was negatively associated with chick age among chicks captured between 30 and 42 days old ($n = 21$; unpubl data). While loon chick age was not known precisely at the time loon chicks were sampled for our study (lakes were visited from 4 to 6 times prior to banding to ascertain hatching status), we estimated chick age on the basis of size and plumage, and timed capture and sampling to coincide with loon chick age of 5–7 weeks as they were sufficiently large to band but still vulnerable to our capture technique (Evers 1993). An examination of chick mass at the time of capture during 1992–2010 shows that mean chick mass increased by 15.8 g per year ($SE = 3.7$) over

that time period. Chick mass was not available for all chicks in the blood Hg data set, but for a smaller data set of 345 chicks from 106 lakes for which both blood Hg and chick mass were known, we found that even after accounting for the effects of chick mass on blood Hg, the trend estimates for the 2 time periods (1992–2000 and 2002–2010) were essentially identical to those estimated for the larger chick blood Hg data set. Thus, changes in chick mass at the time of sampling over the study period do not explain the biphasic trend.

Two laboratories analyzed the blood samples collected for this study, and inter-lab differences could also be a biasing factor. En Chem analyzed samples collected in 1992–2005 whereas Wisconsin State Lab of Hygiene analyzed samples collected in 2006–2010. Both labs used similar analytical procedures and maintained high QA/QC standards (see “Methods”). Because of this, and the fact that samples analyzed at one of the labs spanned the period of the trend reversal, we do not believe that analytical bias contributed to the observed trends.

We can rule out the second factor, increased mercury deposition, because the atmospheric deposition of Hg in the study area decreased monotonically during the time period of interest (see Online Resource 1—Fig. 1a). Likewise, there was a corresponding decline in the concentration of

Hg in lake waters (Online Resource 1—Fig. 1b). Both of these declining time trends have been reported earlier (Watras and Morrison 2008); and the updated data presented here confirm that they continued throughout the loon study period.

The third factor, increased meHg bioaccumulation, seems most likely to account for the bi-phasic trend observed in Wisconsin loons. As shown in a prior study of Little Rock Lake (LRL, a precipitation dominated seepage lake within our loon study area), waterborne meHg and SO₄ both showed a biphasic temporal trend over the 1988–2007 time period (Watras and Morrison 2008). From 1988 to 1998, waterborne meHg declined along with declines in total Hg and SO₄; but, beginning in 1998, the declining trends for meHg and SO₄ were reversed. This reversal occurred when the regional water cycle shifted from a high water regime to a low water regime (Asplund 2008; Asplund and Knight 2009). During the first phase, lake levels were steadily rising; and during the second phase lake levels steadily declined. Since neither total Hg concentration nor SO₄ deposition showed a biphasic time trend, the authors concluded that the in-lake production of meHg was stimulated by the progressive mobilization of SO₄ from exposed littoral sediments as drought conditions worsened.

Previous hydrologic studies in this region have attributed analogous changes in lake-water chemistry to diminished groundwater discharge and (or) increased evapoconcentration during drought (e.g., Kenoyer and Anderson 1989; Wentz et al. 1995; Webster et al. 1996). However, neither of these mechanisms could account for the magnitude of the biphasic changes in SO₄ and meHg that were observed in LRL during 1988–2007. More recent data confirm that waterborne SO₄ concentrations continued to rise for the duration of our loon study (as water levels continued to fall), despite a continued decline in atmospheric SO₄ deposition (Online Resource 1—Fig. 2). The concentration of waterborne meHg has also continued to rise (C.J. Watras, Wisconsin DNR, Boulder Junction, WI, USA, unpublished data).

Numerous studies have demonstrated a link between SO₄ concentration (or loading) and Hg methylation, mediated by changes in the activity of sulfate-reducing bacteria that methylate Hg as a by-product of normal metabolism. However, few studies have extended this link conclusively to fish or piscivorous birds. For our study area, Hrabik and Watras (2002) reported that regional decreases in anthropogenic Hg and SO₄ emissions were associated with a reduction of meHg in lake water and in yellow perch from 1994 to 2000. Although data for yellow perch are not available for the subsequent period 2000–2010, it seems plausible that the observed increases in waterborne SO₄ (Drevnick et al. 2007) and meHg during drought were

associated with a contemporaneous rebound in perch—a preferred prey item for loons (Merrill et al. 2005).

For Minnesota fish, there is evidence of a biphasic pattern of mercury bioaccumulation during this time period. Monson (2009) conducted a regression analysis of the 25-year record of Hg concentrations in standardized length predator fish (walleye [*Sander vitreus*] and northern pike [*Esox lucius*]). A downward trend in Hg concentrations in standard length fish during 1982–1995 reversed and showed an increase during the period 1996–2007. Two studies of fish Hg concentrations in northern Wisconsin have also shown temporal declines (Madsen and Stern 2007; Rasmussen et al. 2007), but these analyses did not search for biphasic patterns and therefore represent an average change in Hg concentrations (0.6% and 0.5% annual decrease for northern Wisconsin walleye, respectively) during the overall period 1982–2005. Our loon study also shows an overall decline in Hg concentrations from 1992 to 2010, similar to the northern Wisconsin walleye Hg data, but shows a biphasic pattern similar to that in the Minnesota fish-Hg study by Monson (2009).

Differences in lake chemistry, growth rates and food web structure may complicate generalizations about the factors governing meHg bioaccumulation. In our loon data, the second best fitting model for adult blood Hg trends included an interaction between pH and year, suggesting that the rate of change in Hg concentration over time depends on lake pH, with more rapid decreases over time in more acidic lakes (Online Resource 1—parameter estimates in Table 3). The interplay between atmospheric deposition, lake chemistry and other factors that affect microbial community composition, activity and trophic transfer (e.g., Watras et al. 2006; Simoneau et al. 2005; Chen and Folt 2005) likely leads to spatial differences in loon blood trends. Nevertheless, our biphasic time trend data for Wisconsin loons are largely consistent on a regional scale with contemporaneous trends in waterborne SO₄ and meHg observed in Little Rock Lake. We caution, however, that Little Rock Lake may not be a suitable analog for all lakes visited by loons within our study region—even though small seepage lakes predominate the local landscape (<http://www.dnr.state.wi.us/>).

Conclusions

A time trend reversal was observed in Wisconsin loon blood Hg concentrations, with a decline occurring from 1992 to 2000 followed by a rise from 2002 to 2010, but with concentrations still below the early 1990s levels. This bi-phasic pattern was similar to trends observed for waterborne meHg and SO₄ in Little Rock Lake, an intensively studied seepage lake within our study area. The latter trends were strongly

associated with changes in the regional water cycle, independent of changes in the atmospheric deposition of Hg or SO₄. We tentatively conclude that drought-induced changes in the aquatic meHg cycle may have rippled through the food chain to loons. But we caution that this effect may have lake-specific and/or region-specific attributes. We also caution that our findings do not discount the importance of anthropogenic emissions or atmospheric deposition of Hg, which has been the primary source of anthropogenic Hg to lakes in northern Wisconsin. Instead, they illustrate how additional factors may exacerbate the bioaccumulation and trophic transfer of meHg ultimately derived from depositional loadings. Therefore, accurate interpretation of long-term Hg trend data requires a comprehensive sampling strategy to measure and integrate information from multiple environmental factors associated with Hg bioaccumulation.

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