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Patterns of Mercury and Selenium Exposure in Minnesota Common Loons

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Abstract: Common loons (*Gavia immer*) are at risk of elevated dietary mercury (Hg) exposure in portions of their breeding range. To assess the level of risk among loons in Minnesota (USA), we investigated loon blood Hg concentrations in breeding lakes across Minnesota. Loon blood Hg concentrations were regressed on predicted Hg concentrations in standardized 12-cm whole-organism yellow perch (*Perca flavescens*), based on fish Hg records from Minnesota lakes, using the US Geological Survey National Descriptive Model for Mercury in Fish. A linear model, incorporating common loon sex, age, body mass, and log-transformed standardized perch Hg concentration representative of each study lake, was associated with 83% of the variability in observed common loon blood Hg concentrations. Loon blood Hg concentration was positively related to standardized perch Hg concentrations; juvenile loons had lower blood Hg concentrations than adult females, and blood Hg concentrations of juveniles increased with body mass. Blood Hg concentrations of all adult common loons and associated standardized prey Hg for all loon capture lakes included in the study were well below proposed thresholds for adverse effects on loon behavior, physiology, survival, and reproductive success. The fish Hg modeling approach provided insights into spatial patterns of dietary Hg exposure risk to common loons across Minnesota. We also determined that loon blood selenium (Se) concentrations were positively correlated with Hg concentration. Average common loon blood Se concentrations exceeded the published provisional threshold. *Environ Toxicol Chem* 2019;38:524–532. Published 2018 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Common loon; Exposure risk; Mercury; Selenium; Wildlife toxicology

INTRODUCTION

Several studies have suggested that common loons (*Gavia immer*) are at risk of elevated dietary mercury (Hg) exposure in portions of their breeding range (reviewed in Evers et al. 2010). Altered adult loon nesting behavior (Evers et al. 2008), reduced productivity (Barr 1986; Meyer et al. 1995, 1998), and altered chick behavior (Nocera and Taylor 1998; Counard 2001) have been associated with elevated Hg exposure. Most of the Hg in fish tissue (≥95%; Wiener and Spry 1996; Wiener et al. 2003; Driscoll et al. 2007) and common loon blood (~87%; Kenow et al. 2015) consists of methylmercury (MeHg). Methylmercury, the most bioavailable and toxic form of Hg, is known to bioaccumulate in fish in contaminated aquatic systems and is readily transferred to piscivorous wildlife such as common loons (Wiener et al. 2003; Burgess and Hobson 2006).

The most important sites of Hg methylation in aquatic systems are anaerobic interfaces in sediments and wetlands where chloride, sulfide, dissolved organic matter, particulates, humic acids, and temperature may affect rates of MeHg formation (reviewed in Wiener et al. 2003). Mercury contamination of fish and wildlife in upper trophic levels tends to occur in "most wetlands, low-alkalinity or low-pH lakes, surface waters with adjoining or upstream terrestrial areas subjected to flooding, and dark-water lakes and streams" (Wiener et al. 2003).

In common loons, exposure to Hg is typically assessed through analysis of blood samples. Blood Hg concentrations reflect relatively recent exposure to dietary Hg (Evers et al. 1998) and consequently, a number of studies have implied a direct relation between Hg exposure in common loons and fish Hg concentrations in the breeding lakes of loons (Meyer et al. 1995, 1998; Evers et al. 1998, 2005, 2008; Scheuhammer et al. 1998; Burgess and Hobson 2006; Burgess and Meyer 2008). Common loon Hg

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exposure was found to vary geographically across the range of the species in North America, with blood Hg concentrations increasing from west to east (Evers et al. 1998). Mean blood Hg concentrations from loons sampled in the Upper Great Lakes region (1.58 ± 0.92 standard deviation [SD] μg/g) were midway between low concentrations in Alaska (USA; 0.66 ± 0.30 μg/g) and the highest concentrations in the Canadian Maritimes (3.53 ± 1.86 μg/g). Loon blood samples in the Upper Great Lakes region assessment included Minnesota samples from the Grand Rapids (MN, USA) area (blood Hg concentrations of 0.50–1.80 μg/g) and Voyageurs National Park (MN, USA; 0.37–2.95 μg Hg/g). Variability in loon blood Hg concentrations among collection sites in the Upper Great Lakes region was attributed to differences in hydrology (fish Hg concentrations were higher than expected in young reservoirs) and lake chemistry (fish Hg concentrations were higher than expected in lakes with low buffering capacity; Evers et al. 1998). Bischoff et al. (2002) observed that livers obtained from common loon carcasses collected in Minnesota contained moderate Hg concentrations relative to that observed in loon livers sampled elsewhere in North America.

Selenium (Se) is an essential trace element in the diets of vertebrates, but it bioaccumulates and can be toxic at elevated concentrations (reviewed in Ohlendorf 2003). Reproductive failure resulting from embryo mortality, developmental abnormalities, reduced juvenile survival, and consequences for the physiology and survival of adults have been related to high levels of Se exposure in aquatic birds (reviewed in Ohlendorf 2003). Selenium may provide a protective effect in reducing toxicity of MeHg in birds because Hg has a strong affinity for Se, forming insoluble Hg–Se complexes in organisms, and these inert bonds may, in some circumstances, provide a level of protection against Hg-induced toxicity (Scheuhammer 1987; Heinz and Hoffman 1998; see review by Khan and Wang 2009). However, elevated levels of Hg and Se together in the maternal diet may have synergistic negative effects on developing embryos and juvenile birds (Heinz and Hoffman 1998).

We assessed the level of risk to Hg exposure among common loons in Minnesota by examining contaminant concentrations in blood collected from loons in breeding lakes across a broad area of Minnesota and relating loon blood Hg concentrations to that of loon prey (standardized fish tissue Hg concentrations) by employing the US Geological Survey (USGS) National Descriptive Model for Mercury in Fish (Wente 2004) and lake characteristics. The modeling approach provided an opportunity to use standardized fish tissue Hg concentration, coupled with published risk assessment screening benchmarks for dietary Hg exposure for common loons, to broaden the spatial extent of our Hg exposure assessment beyond loon capture lakes to Minnesota lakes where fish samples have been collected and analyzed for total Hg. We also examined the risk of Se exposure in Minnesota loons and related this to blood Hg concentrations.

MATERIALS AND METHODS

Collection of blood samples

We captured breeding common loons from lakes in central and northern Minnesota to obtain blood samples during summers in 2010 to 2014 (Supplemental Data, Figure S1). Lakes with loon territories considered for inclusion in the study (Supplemental Data, Table S1) were selected in consultation with Minnesota Department of Natural Resources staff. Loon territories were monitored for nesting activity and breeding success. Adult loons that successfully produced chicks and the resulting juveniles were captured using night-lighting (Evers 1993) or lift-net capture (Kenow et al. 2009) techniques. Each adult loon was marked with a numbered US Fish and Wildlife Service aluminum band and a unique combination of colored leg-bands to aid with field identification of individuals. A blood sample was collected for Hg and Se analyses from the medial metatarsal vein from each adult loon into syringes containing lithium heparin. Body mass and structural measures (body, head, culmen, and tarsus lengths) were also collected. Juvenile loons captured in association with adult capture work were also banded, and a blood sample was similarly collected for Hg and Se analyses. The mean body mass of juvenile loons was 2.48 kg (SD, minimum, and maximum = 0.70, 0.94, and 3.76).

Handling and care of loons were done under approval of the USGS Upper Midwest Environmental Sciences Center Animal Care and Use Committee and complied with the Animal Welfare Act (Public Law 99-198 and 9 CFR Parts 1, 2, and 3). Collections were authorized by US Fish and Wildlife Service Scientific Collecting Permit numbers MB030466-3 and MB123047-1, and Minnesota Department of Natural Resources Special Permit numbers 16508 and 18825.

Fish data

Fish tissue Hg data were obtained from the Minnesota Fish Contaminant Monitoring Program (Minnesota Pollution Control Agency). The data set used in our analysis contained Hg concentrations from various cuts (i.e., whole organism, skin-on fillet, skin-off fillet) totaling 34 035 samples, representing 28 species of fish collected between 1967 and 2014 from lakes across Minnesota. Background details concerning fish collection, processing, and total Hg analysis are provided in Monson (2009).

Water quality data

Water quality data (Secchi depth, pH, total phosphorus, total alkalinity, and chlorophyll-a) for study lakes were obtained from the Minnesota Pollution Control Agency (2015) and the Minnesota Department of Natural Resources Fisheries Unit (H. Rantala, Minnesota Department of Natural Resources, St. Paul, MN, USA, personal communication, 21 December 2015). We used the mean value for each parameter from among data available during the months of May, June, and July in 2011 to 2014 to coincide with the loon capture period. When multiple methods of sample processing were conducted for an individual lake, the median across methods for each water chemistry parameter was used. In a few cases, we used water quality parameter data collected outside of the loon capture period if they were the only data available. Sampling and sample processing were conducted in accordance with Anderson and

Martin (2015) and Swarbrick (2007). The pH for Rabbit Lake (West Portion) was reported by RMB Environmental Laboratories (2011). The trophic state index values for lakes were calculated using the Carlson index (Carlson 1977) and largely obtained from the Minnesota Pollution Control Agency (2017). Area and maximum water depth for lakes were obtained from the Minnesota Pollution Control Agency (2017) website and the Minnesota Department of Natural Resources (2017) LakeFinder website. Lake characteristics of Mantrap Lake were refined by delineation of that portion of the lake that contained territories of loons included in the study, and Loon Marsh physical and chemical parameters were provided by personnel at the Tamarac National Wildlife Refuge (Rochert, MN, USA).

Laboratory analyses

Common loon blood samples were homogenized, digested, and analyzed for Se by inductively coupled plasma–mass spectrometry in accordance with Eurofins SOP EFGS-054, a modified version of US Environmental Protection Agency (USEPA) method 1638 (US Environmental Protection Agency 1996). Samples for total Hg analysis were prepared and analyzed by flow injection atomic fluorescence spectrometry in accordance with USEPA method 1631B (US Environmental Protection Agency 1999). Preparation blanks, quality control samples, matrix spikes, and duplicate samples were analyzed concurrently with samples.

Liquid spikes, including multi-analyte standards, were prepared from certified source standards (CPI Thorium 1000 μg/mL; Inorganic Ventures catalog numbers EFGS-PREPSPIKE1, EFGS-PREPSPIKE2, and CGHG1-1, −2, and −5; High-Purity Standards part numbers 100033-1 and SM-1411-002). The average recovery rate for Se for matrix spikes was 119.6 ± 1.6%, and the relative percentage difference (RPD) for replicate samples was 5.9 ± 0.9%. Mean reporting limits were 0.11 μg/g for Hg and 0.40 μg/g for Se. The average recovery rate for matrix spikes for total Hg was 101.6 ± 0.8% (standard error), and the RPD for replicate measures of total Hg was 1.8 ± 0.5%.

Statistical analyses

We used the fish Hg model described by Wente (2004) to estimate Hg concentrations in a standardized, 12-cm whole-organism yellow perch (*Perca flavescens*) from lakes in Minnesota, using the large state-wide fish tissue Hg data set. Yellow perch are considered a favored prey item of common loons (Evers et al. 2010), and the 12-cm length associated with this standardized perch Hg estimate was based on the size range (10–15 cm; Barr 1996) observed to be most frequently consumed by common loons (Depew et al. 2013). Mercury concentrations vary among fish species (increasing with trophic level), fish size, and species cut (i.e., fillet with skin, fillet without skin, or whole organism for each species) within species (Wente 2004). The Wente model allows for estimation of Hg concentration of a standardized cut of a particular fish species based on the size-species relation with all other fish species. Briefly, the Wente model estimates species-cut relationships between Hg concentration (ppb) and fish length, intercepts are permitted to vary by lake-sampling event combinations, "species cut" defines the sample matrix, and censoring associated with below-detection values is addressed. Specific cuts were based on Minnesota state sampling protocols. Fish Hg concentration and fish lengths enter the model as *ln*(length+1) transformed variables. To improve estimation, we omitted specific sampling events and species cuts with <5 samples. Sampling events associated with loon study lakes were not excluded from the analysis even if <5 samples were present. Regression models were fitted using the cenreg function in the Nondetects And Data Analysis for Environmental Data (NADA) package for the R statistical software system (Lopaka, 2013). Residuals from the model were analyzed to examine model fit for yellow perch (Dalgaard 2008). Model fit was also evaluated using the prediction error (*PE*) estimated from the scale parameter (Wente 2004). The data used for model analysis and model code are available at USGS repositories (US Geological Survey 2017).

To characterize the association of prey Hg with lake characteristics, the correlations between lake metrics/water quality observations and the standardized perch Hg data were estimated using the Spearman rank correlation statistic; confidence intervals (CIs) on correlation estimates were estimated using Fisher's *z* transformation (Fisher 1970).

The *ln* concentration of Hg (ppb) in loon blood was modeled using a linear model (Dalgaard 2008) in R (R Development Core Team 2015) and permitted to vary with the association of *ln* tissue concentration of perch Hg. Means were permitted to vary by age–sex combinations (i.e., adult female, adult male, juvenile). Loon mass (kg) associations were permitted to vary with age (juvenile/adult). The model's fit was examined using residual plots. Data were plotted using the *Graphics of Grammar* (*ggplot2*) package in R (Wickham 2009), which is based on theory developed by Wilkinson (2006).

Selenium was analyzed using a linear model. The *ln*(Se) was predicted by an intercept for each loon sex and a slope estimated for loon mass, with mass allowed to vary for each age group of loons (juvenile and adults). Although we addressed censoring of Hg values statistically (see above), such censoring was rare for Se (1 of 74 and 5 of 50 juvenile and adult loons, respectively). Accordingly, we substituted one-half the reporting limit (i.e., 0.2 μg/g) for censored Se values. The molar concentrations of both Se and Hg were used to estimate Hg/Se molar ratios. The Hg/Se molar ratios were analyzed using the same linear model as the loon blood Se model.

RESULTS

Blood samples collected from 124 (31 adult females, 42 adult males, 1 adult unknown, 50 juveniles) common loons in Minnesota were used in the analyses. Blood Hg concentrations averaged 0.812 μg/g (SD, minimum, and maximum = 0.402, 0.236, and 1.860 μg/g, respectively) in adult females, 1.075 μg/g (SD, minimum, and maximum = 0.535, 0.307, and 2.260, respectively) in adult males, and 0.151 μg/g (SD, minimum, and maximum = 0.162, 0.010, 0.708) in juveniles (Table 1). There was

TABLE 1: Average (minimum, maximum) mercury (Hg) concentration, selenium (Se) concentration, and Hg/Se molar ratios of breeding adult and juvenile common loon blood samples collected across Minnesota, USA, 2010–2014

	Adult female ($n = 31$)	Adult male ($n = 42$)	Juvenile ($n = 50$)
Blood Hg concentration (μg/g)	0.81 (0.24, 1.86)	1.08 (0.31, 2.26)	0.15 (0.01, 0.71)
Blood Se concentration (μg/g)	1.60 (0.52, 3.44)	1.11 (0.20, 2.63)	0.67 (0.20, 1.35)
Blood Hg/Se molar ratio	0.23 (0.06, 0.66)	0.44 (0.11, 1.84)	0.09 (0.01, 0.66)

no clear pattern in adult blood Hg concentrations across the state (Supplemental Data, Figure S1).

Standardized 12-cm whole-organism perch Hg concentration estimates were based on 34 035 measures of fish Hg from 870 Minnesota lakes (including most of the study lakes) representing 47 species-cut combination sample types from 2385 sampling events. Model-predicted standardized perch *ln* Hg concentration were compared with actual perch *ln* Hg concentration values. We selected perch from the dataset that were within ±1 cm of the 12-cm standardized length for this evaluation, yielding 53 perch 11 to 13 cm in length that were sampled among 26 lakes. The relation indicated a strong linear relation and good model performance (slope = 1.09 [95% CI = 0.90, 1.27], intercept = –0.28 [95% CI = –1, 0.45]; adjusted $r^2 = 0.73$).

Standardized perch Hg concentration was negatively correlated with pH of loon study lakes ($r = –0.44$, $p = 0.04$, 95% CI = –0.72, –0.02). Otherwise, estimated correlations between standardized perch Hg concentration and other lake characteristics were small in magnitude (all correlation point estimates ≤ 0.26, $p \geq 0.22$).

The variables sex, age, body mass, and standardized perch Hg concentration representative of each study lake were associated with 83% of the variability in common loon blood Hg concentrations ($F_{5,105} = 108$, $p < 0.001$, adjusted $r^2 = 0.83$, $PE = 34.16\%$). The following associations derive from this model. Loon blood Hg concentration was positively associated with standardized perch Hg (97% change/unit *ln*[perch Hg concentration], 95% CI = 53%, 154%; Figure 1). Adult male and female blood Hg concentrations were not convincingly different (26%, 95% CI = –32%, 135%). Juvenile blood Hg concentrations were substantially lower than adult female concentrations (–98%, 95% CI = –100%, –84%). The relation between blood Hg concentration and body mass was weak for adult (6% change/kg, 95% CI = –43%, 94%), but estimated mean juvenile loon blood Hg concentration increased by 154% (95% CI = 103%, 217%) per 1-kg increase in body mass (Figure 2).

Blood Se concentrations averaged 1.60 μg/g (SD, minimum, and maximum = 0.79, 0.52, and 3.44, respectively) in adult females, 1.11 μg/g (SD, minimum, and maximum = 0.53, 0.2 [default for below detection level], 2.63, respectively) in adult

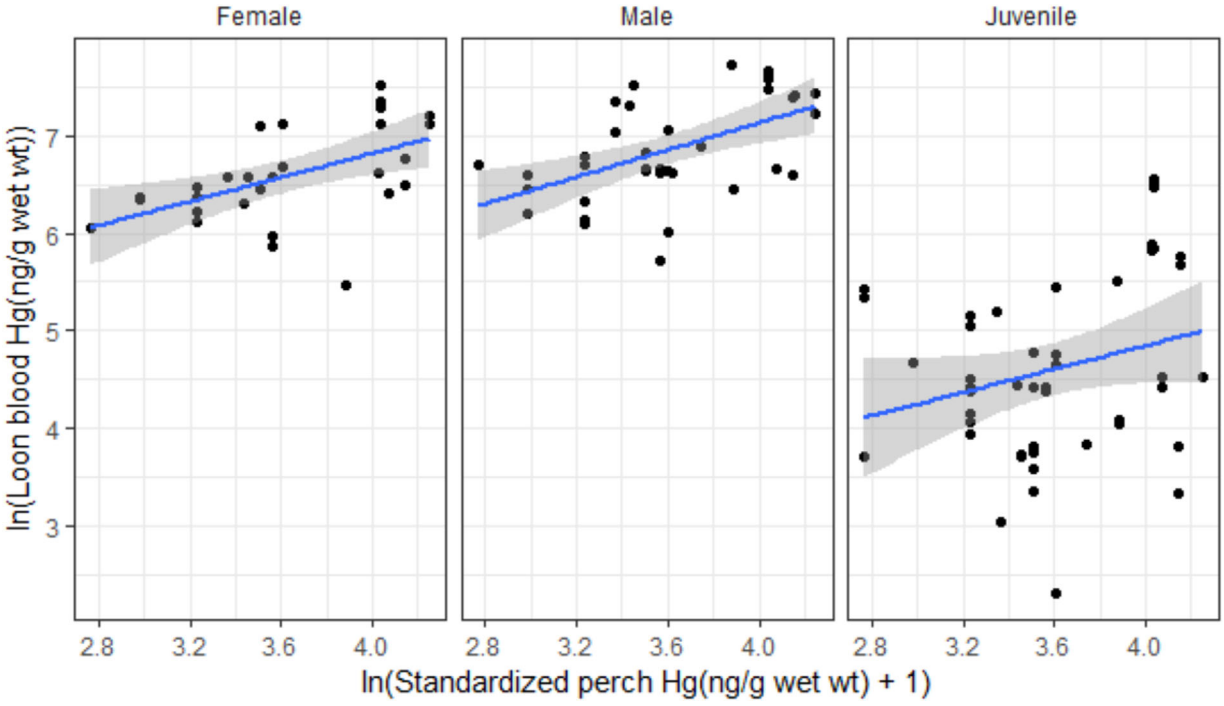

FIGURE 1: Estimated associations (with 95% confidence intervals) between log-transformed common loon blood mercury (Hg) concentrations and log-transformed standardized yellow perch mercury concentrations in blood from adult female (intercept = 4.35 [95% CI 2.87–5.84], slope = 0.62 [95% CI 0.21–1.02], adjusted $r^2 = 0.26$), adult male (intercept = 4.35 [95% CI 2.96–5.75], slope = 0.69 [95% CI 0.31 to 1.08], adjusted $r^2 = 0.24$), and juvenile loons (intercept = 2.44 [95% CI 0.04–4.85], slope = 0.60 [95% CI −0.07–1.27], adjusted $r^2 = 0.05$) from Minnesota, USA.

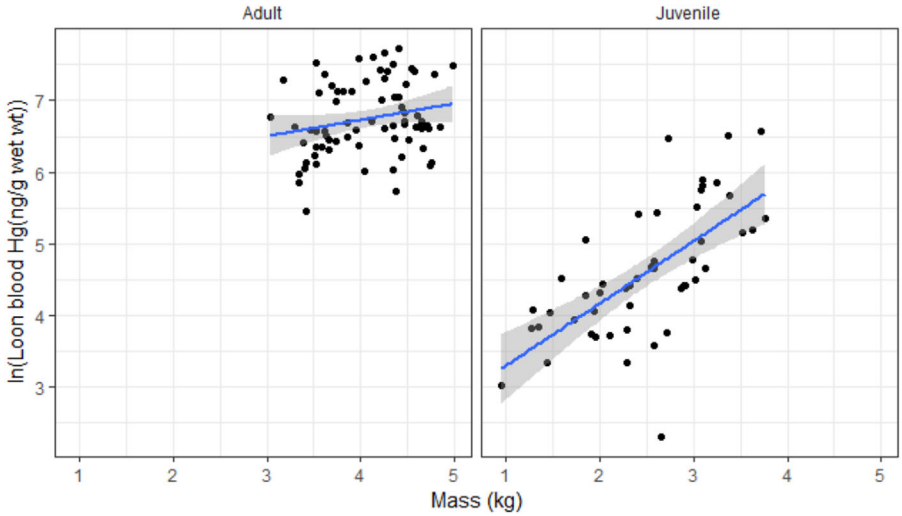

FIGURE 2: Plot of the relation of blood mercury (Hg) concentration and body mass of Minnesota (USA) common loons. The lines are the marginal slope estimates of adult (intercept = 5.81 [95% CI 4.82–6.80], slope = 0.23 [95% CI −0.01–0.47], adjusted $r^2 = 0.03$) and juvenile (intercept = 2.44 [95% CI 1.71–3.18], slope = 0.87 [95% CI 0.58–1.15], adjusted $r^2 = 0.42$) loons, and the shaded regions represent 95% confidence intervals.

males, and 0.67 μg/g (SD, minimum, and maximum = 0.25, 0.2, 1.35) in juveniles. Loon blood Hg was positively correlated with Se concentration ($r = 0.54$, 95% CI = 0.22, 0.76; Figure 3). Mean blood Se concentrations of adult males and juveniles were not statistically different from that of females (i.e., relative differences were –27%, 95% CI = –55%, 17%) and –59%, 95% CI = –93%, 151%), respectively. No strong associations were evident between adult (–3% [95% CI = –41%, 51%] change per kg) or juvenile (–2% [95% CI = –20%, 19%] change per kg) loon body mass and *ln* blood Se concentration.

Blood Hg/Se molar ratios averaged 0.23 (minimum and maximum = 0.06 and 0.66) in adult females, 0.44 (minimum and maximum = 0.11 and 1.84) in adult males, and 0.09 (minimum and maximum = 0.01 and 0.66) in juveniles. Mean Hg/Se molar ratios were relatively smaller for males than females (31%, 95% CI = 7%, 62%) but statistically not different for adult females and juveniles (–44%, 95% CI = –74%, 21%). Estimated mean juvenile loon blood Hg/Se molar ratio increased by 8% (95% CI = 0%, 17%) per 1-kg increase in body mass. The associations between Hg/Se molar ratios and body mass for adult loons were weak

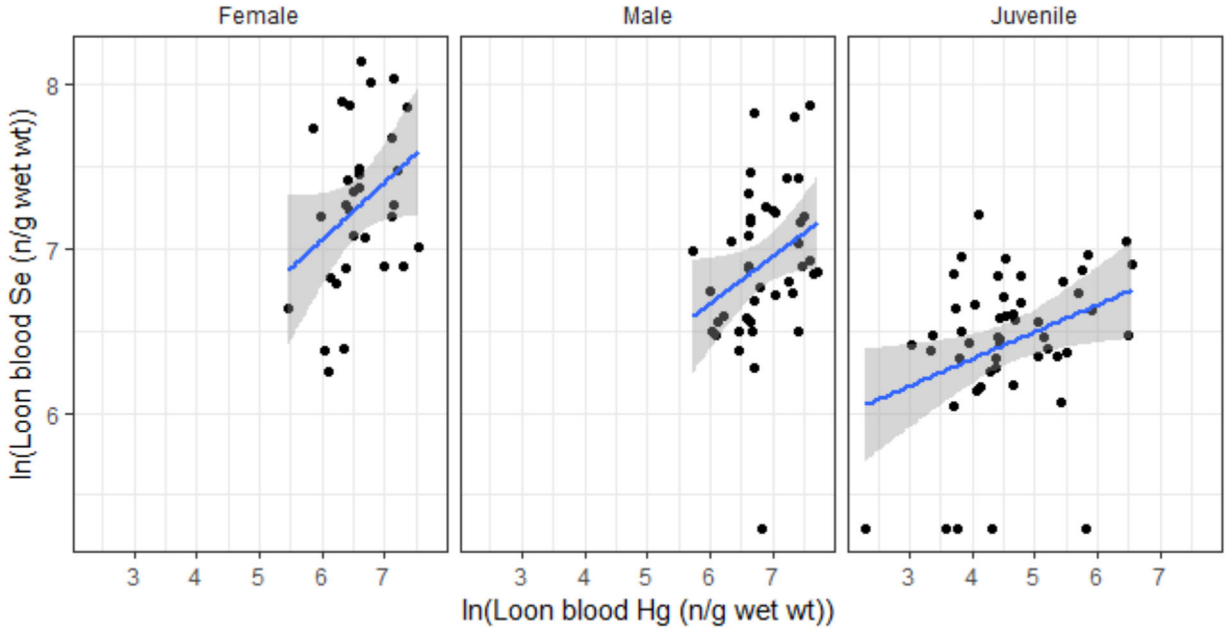

FIGURE 3: The relation of selenium (Se) and blood mercury (Hg) concentrations in Minnesota (USA) common loon blood by age and sex (male: intercept = 4.35 [95% CI 2.96–5.75], slope = 0.69 [95% CI 0.31–1.08], adjusted $r^2 = 0.24$; female: intercept = 4.35 [95% CI 2.87–5.84], slope = 0.62 [95% CI 0.21–1.02], adjusted $r^2 = 0.26$; juvenile: intercept = 2.44 [95% CI −0.04–4.85], slope = 0.60 [95% CI −0.07–1.27], adjusted $r^2 = 0.05$). The lines are the marginal slope estimates, and the shaded regions represent 95% confidence intervals.

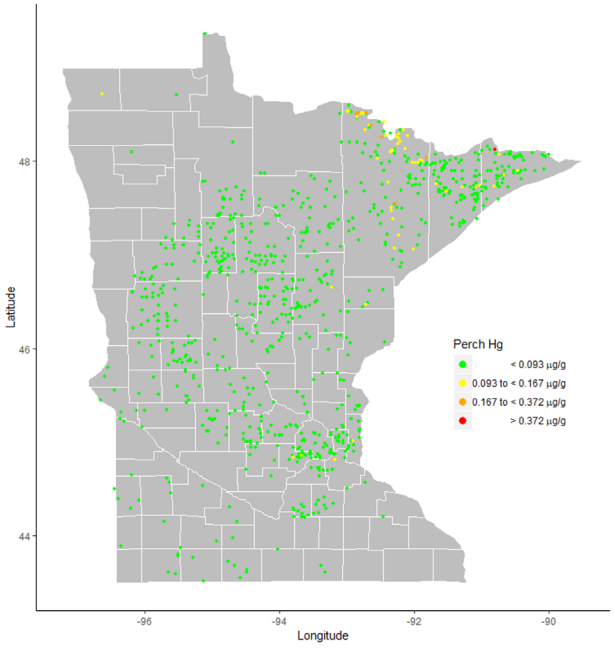

FIGURE 4: Predicted standardized 12-cm whole-organism yellow perch mercury (Hg) concentration for Minnesota, USA lakes where fish tissue Hg data were obtained from the Minnesota Fish Contaminant Monitoring Program; predictions were derived from the Wente (2004) fish Hg model. Perch Hg values are categorized according to thresholds for dietary methylmercury exposure in prey fish. The benchmarks reflect thresholds for adverse behavioral impacts in adult loons and no-observed-adverse-effect level in captive loon chicks (0.093 µg Hg/g), significant reproductive impairment (0.167 µg Hg/g), and reproductive failure (0.372 µg Hg/g).

(–7%, 95% CI = –24%, 15%; change per kg). Associations between Hg/Se molar ratios and loon body mass for juveniles and adults were 8% (95% CI = 0%, 17%) and –7% (95% CI = –24%, 15%) per 1-kg increase in body mass.

Figure 4 illustrates the distribution of lakes where fish tissue Hg data were obtained from the Minnesota Fish Contaminant Monitoring Program and categorical standardized perch values derived from the Wente (2004) fish Hg model. Perch Hg values are categorized according to thresholds for dietary methylmercury exposure in prey fish. The benchmarks (Evers et al. 2011) reflect thresholds for adverse behavioral impacts in adult loons and no-observed-adverse-effect level (NOAEL) in captive loon chicks (0.093 µg Hg/g), significant reproductive impairment (0.167 µg Hg/g), and reproductive failure (0.372 µg Hg/g).

DISCUSSION

The mean blood Hg concentrations among adult common loons sampled in the present study were, on average, considerably lower than those reported by Evers et al. (1998) for common loons within the Great Lakes region and across North America. Blood Hg concentrations of all the adult common loons we sampled fell well below an established blood Hg threshold for adult loons of 3.0 µg/g (wet wt) for adverse effects on behavior, physiology, survival, and reproductive success (Evers et al. 2008, 2011). Whereas 3.0 µg/g Hg is generally recognized as the lowest-observed-adverse-effect level for common loons, the suggested NOAEL is 1.0 µg/g (Evers et al. 2011). In our study, 65% of adult loons had blood Hg concentrations below the NOAEL, and 35% fell within the 1- to 3-µg/g Hg range, where effects remain undefined.

Conditional mean Hg concentrations in adult male loons in the present study may have been higher (26%) than those in adult females (Figure 1), but confidence limits were quite wide. According to Evers et al. (1998), blood Hg concentrations in adult male common loons from selected sites across North America averaged 19% (range 12–45%) higher than females. Burgess and Meyer (2008) determined that mean blood Hg concentration, adjusted for lake pH, in male common loons was 25% higher than in females in Wisconsin and the Canadian Maritimes.

Juvenile loon blood Hg levels in our study, adjusted for body mass, were significantly lower than that of adults (Figure 1). Evers et al. (1998) and Fevold et al. (2003) reported that blood Hg of loon offspring significantly correlated with adult blood Hg across North America ($r^2 = 0.56$ with adult females, $r^2 = 0.48$ with adult males) and on Wisconsin study lakes ($r^2 = 0.52$ with adult females, $r^2 = 0.61$ with adult males), respectively. Mean blood Hg of adults was 10 times that of 3- to 6-wk-old juveniles (Evers et al. 1998). Our results are not directly comparable to the juvenile blood Hg concentrations reported across North America study locations (including the Grand Rapids and Voyageurs National Park study areas) by Evers et al. (1998), because the age of juveniles of our study varied from approximately 3 to 10 wk. Fevold et al. (2003) determined that mean blood Hg concentrations in Wisconsin loon chicks increased by 17.5% between 2 and 5 wk of age, and hence the importance of accounting for body mass, as in our study. Our model indicated a 154% increase in blood Hg concentration of juvenile loons/kg increase in body mass. Increasing blood Hg concentration in aging juveniles is likely a function of 1) exploitation of larger prey with age, and hence prey containing higher Hg concentration (Wiener and Spry 1996); and 2) a reduction in Hg depuration to feathers as feather development ceases (Fournier et al. 2002; Kenow et al. 2007).

We observed a strong relation between adult and juvenile common loon Hg exposure and estimated standardized perch Hg concentration for the 24 Minnesota lakes included in the present study (Figure 1). Previous studies have documented strong positive correlations between Hg concentrations of prey fish samples collected from study lakes and loon (Scheuhammer et al. 1998; Burgess and Hobson 2006; Champoux et al. 2006; Burgess and Meyer 2008) and grebe (Ackerman et al. 2015) blood Hg concentrations. Scheuhammer et al. (1998) found positive correlations between adult (slope = 1.29, $r = 0.70$) and juvenile (slope = 0.68, $r = 0.54$) loon blood Hg and prey fish Hg concentrations among south-central Ontario, Canada study lakes. Champoux et al. (2006, Figure 2) illustrate a similar pattern to that observed in our study with respect to the relative relations of adult male, adult female, and juvenile loon blood Hg concentrations to prey fish Hg levels among study lakes in Québec, Canada. Burgess and Meyer (2008) documented a

similar outcome: loon blood Hg concentrations increased with elevated prey fish Hg concentrations in lakes in Maritime Canada and Wisconsin (slope = 1.00, $r^2 = 0.87$). Ackerman et al. (2015) estimated grebe blood Hg as a function of prey fish collected in study lakes in California lakes and reservoirs, day of year, lake size and shape, and lake elevation (slope of the relation between prey fish and grebe blood Hg = 0.706).

Standardized perch Hg concentration was inversely correlated with lake pH in our study ($r^2 = -0.44$). This finding is consistent with other investigations of the relation between fish Hg concentration and lake physicochemical characteristics (Cope et al. 1990; Grieb et al. 1990; Suns and Hitchin 1990; Greenfield et al. 2001; Wiener et al. 2003; Burgess and Meyer 2008). Lake pH was the best predictor of Hg concentrations in yellow perch among lake characteristics in studies by Suns and Hitchin (1990) and Greenfield et al. (2001). Low-pH lakes are among the known Hg-sensitive ecosystems (Wiener et al. 2003) where relatively high rates of methylation and MeHg trophic transfer may occur. Several studies have observed that adult and juvenile common loon Hg exposures were inversely related to lake pH (Meyer et al. 1995, 1998; Fevold et al. 2003; Burgess and Meyer 2008).

The risk assessment screening benchmarks for dietary MeHg exposure for common loons proposed by Depew et al. (2012), based on a synthesis of the relevant literature (Barr 1986; Evers et al. 2004, 2008; Burgess and Meyer 2008; Kenow et al. 2011), were 0.1, 0.18, and 0.4 μg MeHg/g wet weight in prey fish. The dietary benchmarks reflect thresholds for adverse behavioral impacts in adult loons and NOAEL values in captive loon chicks (0.1 μg MeHg/g), significant reproductive impairment (0.18 μg MeHg/g), and reproductive failure (0.4 μg MeHg/g). The standardized perch Hg concentrations representative for 24 loon lakes included in the fish model from the present study varied from 0.015 to 0.069 μg Hg/g. Standardized prey Hg for all lakes included in our study were less than the 0.1 μg MeHg/g (0.093 μg Hg/g equivalent) benchmark proposed by Depew et al. (2012) as a threshold for adverse behavioral impacts in adult loons.

The application of the Wente (2004) fish Hg model to estimate Hg concentrations in a standardized yellow perch provided a useful means to account for variability observed in Hg exposure among adult and juvenile common loons in Minnesota. The modeling approach also provided an opportunity to use standardized perch Hg to assess the spatial distribution of Hg in typical loon forage fish across Minnesota, where an adequate number of fish samples have been collected and analyzed for total Hg (Figure 4). Of the 870 lakes included in the state-wide analysis, predicted values for one lake (Larch Lake, Cook County) exceeded the prey Hg threshold (0.37 μg Hg/g equivalent) for reproductive failure in common loons, and an additional 7 lakes (all within St. Louis County) exceeded the threshold (0.167 μg Hg/g equivalent) proposed for significant loon reproductive impairment. This area coincides with a portion of Minnesota identified among biological Hg hotspots (with predicted male loon blood Hg $>$ 2 μg/g) concentrated in northeastern Minnesota by Evers et al. (2011, Figures 2 and 3). An additional 56 lakes met the benchmark (0.093 μg Hg/g equivalent) for adverse behavioral impacts in adult loons; most of these lakes are in northeastern Minnesota, primarily in St. Louis County. These inferences need to be interpreted in light of predictor uncertainty. However, calculation of this uncertainty for individual lakes from the Wente model may represent a research question (G. Schwarz, US Geological Survey, Reston, VA, personal communication, 19 April 2018; cf. Christensen et al. 2006; Depew et al. 2013).

Wiener et al. (2012) estimated risk for common loons among lakes in the Great Lakes region based on Hg in samples of yellow perch in the $<$19-cm length range (using some of the same Minnesota data that was incorporated into our analysis), and concluded that mean whole-body concentrations exceeded a dietary threshold (0.16 μg Hg/g equivalent) for significant reproductive effects in loons in 7.3% of waters included in the study and that 0.8% of sites exceeded a dietary threshold (0.41 μg Hg/g) associated with complete reproductive failure in common loons. Our standardized perch Hg approach to evaluating Hg-exposure risk to common loons suggests that an overall lower proportion of lakes in Minnesota pose a dietary Hg risk for significant reproductive effects in common loons (8 of 870 lakes, 0.9%; illustrated in Figure 4) relative to lakes within the Wiener et al. (2012) Great Lakes region analysis (7.3%). Differences in these percentages may be attributed to 1) the use of larger perch, up to 19 cm in length compared with the standardized 12-cm perch used in our analysis, with larger prey being expected to contain higher concentrations of Hg (Wiener and Spry, 1996); and/or(2) the result of sampling in the western portion of the Great Lakes Region where region-wide spatial trends of increasing fish Hg concentration from west to east have been documented (Monson et al. 2011). Note that the dietary threshold values proposed by Depew et al. (2012) and Wiener et al. (2012) differ slightly (0.167 μg Hg/g equivalent vs 0.16 μg Hg/g, respectively).

Average common loon blood Se concentrations in the present study exceeded the provisional threshold warranting further study of 1.0 μg Se/g wet weight (Ohlendorf and Heinz 2011). However, several studies have documented higher blood Se concentrations in healthy marine birds (see review in Ohlendorf and Heinz 2011); due to the variable findings among species, those authors concluded that blood Se concentrations cannot be conclusively related to reproductive or health effects. For comparison, blood Se concentrations in adult female loons breeding in Wisconsin at the time of egg laying averaged 2.42 μg/g wet weight, with minimum and maximum concentrations of 1.39 and 4.55 μg Se/g, respectively (Kenow et al. 2015).

We found no clear evidence to suggest that Hg or Se exposure among the common loons sampled in the present study were at levels considered to adversely affect behavior, reproduction, or survival. However, our sampling did not include lakes in northeastern Minnesota identified as potentially having elevated Hg concentrations in loon prey fish associated with adverse behavioral impacts and/or significant reproductive impairment in adult loons. Future evaluations of Hg exposure in piscivorous wildlife may want to incorporate estimated standardized perch Hg concentrations in a stratified sample design.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4331.

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Data Accessibility—Data, associated metadata, and calculation tools are available from the corresponding author (kkenow@usgs.gov).

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