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INFLUENCE OF IN OVO MERCURY EXPOSURE, LAKE ACIDITY, AND OTHER FACTORS ON COMMON LOON EGG AND CHICK QUALITY IN WISCONSIN

KEVIN P. KENOW,*† MICHAEL W. MEYER,‡ RONALD ROSSMANN,§ BRIAN R. GRAY,† and MICHAEL T. ARTS‖
†Upper Midwest Environmental Sciences Center, US Geological Survey, La Crosse, Wisconsin, USA
‡Wisconsin Department of Natural Resources, Rhinelander, Wisconsin, USA
§Large Lakes Research Station, US Environmental Protection Agency, Grosse Ile, Michigan, USA
‖Department of Chemistry and Biology, Ryerson University, Toronto, Ontario, Canada

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Abstract: A field study was conducted in Wisconsin (USA) to characterize in ovo mercury (Hg) exposure in common loons (*Gavia immer*). Total Hg mass fractions ranged from 0.17 μg/g to 1.23 μg/g wet weight in eggs collected from nests on lakes representing a wide range of pH (5.0–8.1) and were modeled as a function of maternal loon Hg exposure and egg laying order. Blood total Hg mass fractions in a sample of loon chicks ranged from 0.84 μg/g to 3.86 μg/g wet weight at hatch. Factors other than mercury exposure that may have persistent consequences on development of chicks from eggs collected on low-pH lakes (i.e., egg selenium, calcium, and fatty acid mass fractions) do not seem to be contributing to reported differences in loon chick quality as a function of lake pH. However, it was observed that adult male loons holding territories on neutral-pH lakes were larger on average than those occupying territories on low-pH lakes. Differences in adult body size of common loons holding territories on neutral-versus low-pH lakes may have genetic implications for differences in lake-source-related quality (i.e., size) in chicks. The tendency for high in ovo Hg exposure and smaller adult male size to co-occur in low-pH lakes complicates the interpretation of the relative contributions of each to resulting chick quality. *Environ Toxicol Chem* 2015;34:1870–1880. © 2015 SETAC

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INTRODUCTION

Several studies have documented reduced reproductive performance in common loons (*Gavia immer*) nesting on acidic lakes (see review by Evers et al. [1]). There has been speculation that reduced diversity and abundance of prey on low-pH lakes results in low foraging success for common loons, with consequences on breeding success ([2,3]; but see Parker [4]), although Burgess and Meyer [5] concluded that the evidence in support of this assertion was not convincing. Increased bioavailability of methylmercury (MeHg) is also associated with acid lakes [6], resulting in elevated levels of MeHg in fish [7], the primary prey of loons. Common loons are thought to be sensitive to the toxic effects of MeHg [7–9], and investigations have indicated that loons nesting on low-pH lakes have elevated mercury (Hg) levels in their blood and eggs [10–15]. A portion of the maternal body burden of MeHg is transferred to eggs during egg formation [16] and is subsequently available to the developing embryo, which is considered a life stage that is much more sensitive than the adult stage to MeHg exposure [17].

A captive rearing study indicated that common loon chicks hatched from eggs collected from acidic lakes in Wisconsin have elevated Hg exposure at hatch and that the quality of these birds may be compromised regardless of subsequent dietary Hg intake or forage availability. Chicks from low-pH lakes exhibit lower growth rates [18], behavioral differences (less motivated, lethargic [19]), and evidence of lymphoid depletion [20] relative to chicks from neutral-pH lakes. These lake-source effects observed in the laboratory suggest that in ovo exposure to MeHg or other factors related to lake pH may have consequences on chick development and quality. Mortality, reduction in growth, and developmental abnormalities have been documented in developing embryos of mallard eggs externally treated with MeHg [21]. The effects of in ovo MeHg exposure in mallard ducklings persist beyond the embryo to include effects on behavior and survival and on the frequency of brain lesions [22,23].

In a field study, we assessed the level of in ovo MeHg exposure that resulted in detrimental effects on quality and survival of common loon embryos and resulting chicks [24]. We found a dose-dependent reduction in hatchability in eggs injected with various doses of MeHg (median lethal concentration [LC50] = 1.78 μg Hg/g wet wt), a negative relation between yolk sac mass and egg total mercury content, decreased responsiveness to a frightening stimulus in chicks with relatively high in ovo exposure to MeHg, and increased length of incubation period ($r = 0.75$, $p < 0.0001$, $n = 23$) with increasing egg mercury mass fraction. These differences could have long-term implications for survival. For example, Piper et al. [25] found that survival of juvenile loons to 3 yr (based on resighting of marked individuals) was positively associated with both pH and size of natal lakes in northern Wisconsin.

Alternatively, other factors related to lake pH, such as altered availability of toxic metals (e.g., lead, cadmium, aluminum; [26–28]) or essential elements (e.g., calcium [Ca], phosphorus; [27,28]), may have persistent consequences on the development of chicks that hatch from eggs laid by loons resident on low-pH lakes.

Differences in egg size and composition, a consequence of maternal quality, also affect chick quality. Egg size can vary considerably among females, and large eggs generally produce larger hatchlings than those produced from smaller eggs [29–32]. This phenomenon has been observed in common loons

* Address correspondence to kkenow@usgs.gov.
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(K.P. Kenow et al., unpublished data), and the effects last through fledging [32]. Egg size may reflect transitory attributes of the female such as age, experience, and/or body condition [32]. Effects of parental age on egg laying (size and egg composition) are discussed in Bogdanova et al. [33]. Anderson and Alisauskas [34] provided a comprehensive review of the relation of egg size to higher mass in ducklings, more lipid, greater thermal resistance, rapid growth, and greater motor performance. There is precedence in common loon egg volume differences in relation to lake pH. For example, Pollentier et al. [35] reported that average egg volume and egg length in common loons are reduced by 4% to 5% and by 2% to 3%, respectively, in low-pH compared with neutral-pH lakes. In support of this finding, Kenow et al. [18] reported that common loon chicks at hatch from low-pH lakes tended to be approximately 3.5% smaller than chicks from neutral-pH lakes.

Chick development also depends on sufficient lipid of the appropriate composition from the yolk and on metabolic ability for growth and differentiation of the embryo and early chick nutrition [36]. Fatty acids derived from these lipids are assembled in the maternal liver [36] and reflect the diet of the female during the period of egg formation [37]. Consequently, we were interested in examining differences in fatty acid composition in eggs from low- versus neutral-pH lakes. Of particular interest were essential fatty acids. The essential fatty acids are fatty acids that 1) cannot be synthesized at all by birds, including α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), and 2) cannot normally be synthesized in amounts sufficient to maintain birds in a state of optimal physiological functioning/nutrition (including eicosapentaenoic [20:5n-3], docosahexaenoic [22:6n-3], and arachidonic [20:4n-6]) acids. The essential fatty acids docosahexaenoic and arachidonic are crucial for neural development and health [38], and docosahexaenoic acid plays a critical role in the development of the retina and maintenance of visual acuity in vertebrates [39,40]. Furthermore, linoleic acid and α-linolenic acid may be converted to arachidonic acid and eicosapentaenoic acid+docosahexaenoic acid, respectively, in the developing embryo [41,42]. Consequently, we characterized all 5 fatty acids as essential fatty acids.

In the present study we report on the results from a field study aimed at characterizing in ovo MeHg exposure in common loons in northern Wisconsin (USA) and assessing other factors that may have persistent consequences on the development of chicks from eggs collected on low-pH lakes. Egg quality measures of mass fractions of Hg, selenium (Se), and Ca, volume, and fatty acid composition are evaluated with respect to characteristics of natal lakes and maternal influences. Body size differences of adults occupying territories on low- versus neutral-pH lakes are examined. We also report blood Hg levels at hatch and evaluate the differences in mass fractions of Hg among chick tissues.

MATERIALS AND METHODS

Common loon nesting activity was monitored in 5 counties (Iron, Vilas, Oneida, Lincoln, and Price Counties) in north-central Wisconsin during the 2005 and 2006 breeding seasons. Our study lakes, which all supported breeding loons, ranged from the lowest known pH in the area to neutral-pH lakes. Timing of the initiation of egg laying was determined within 1 d. Eggs (normally 2 per nest) were measured (length and width to the nearest 0.01 mm), and labeled with a Sharpie pen to aid in later identification of the first and second egg laid.

We collected eggs (usually 2 per nest) from a sample of loon nests ($n = 20$ from among 18 lakes) within a few days of laying and preserved egg contents in a certified clean sample container (EP Scientific Products QA Level 1) by freezing ($\leq -20\,^{\circ}C$) until submission for laboratory analyses (see *Laboratory analyses*) for total Hg, MeHg, Al, Pb, Se, Cd, and Ca. Egg volume was determined volumetrically by displacement or by using Archimedes' principal for eggs weighed in both air and water. At the time of egg collection we captured adult female loons from the nest (maternal loons), usually at night (as in Kenow et al. [43]), and obtained a blood sample for Hg, MeHg, Al, Pb, Se, Cd, and Ca analyses. Territorial adults were also captured in the spring using a lift-net technique [43]. An electronic hanging balance was used to determine body mass to the nearest 0.01 kg. Body measures, including head length, culmen length, and body length without tail, were obtained following the guidelines of Baldwin et al. [44]. Head and culmen length were determined to the nearest 0.01 mm using a digital caliper. Head length was measured along the midline of the skull between the tip of the bill and the external occipital protuberance of the supraoccipital bone at the rear of the skull. Culmen length was determined by measuring the length of the exposed bill along its anterior midline between the tip of the bill and the base of the upper mandible where the skin and feathers of the forehead begin. Body length represents the outstretched length of the loon in ventral recumbency as measured from the anterior tip of the bill to the posterior tip of the pygostyle.

A second sample of loon nests was monitored where eggs remained in the nest and incubated by adults for approximately 23 d before collection ($n = 16$ from among 14 lakes). These eggs were transported to a nearby field laboratory in coolers warmed to approximately 37 °C, where they were artificially incubated in commercial incubators until hatch. After chicks had hatched and were completely dry, they were weighed and measured for length of culmen, head, tarsus, wing, and body. We collected a blood sample (~1 mL) and sacrificed the chicks to obtain Hg residue mass fractions for selected tissues (liver, kidney, brain, muscle, feather, yolk sac, and carcass [remaining tissue following harvest of organs]).

Additional samples of common loon eggs ($n = 24$) were collected within 1 d of being laid during the 2007 breeding season for fatty acid analysis. One egg (second egg laid) was collected from each of 24 territories on 18 lakes. Homogenized egg contents were frozen in liquid nitrogen and then stored at –80 °C until submission for fatty acid analysis.

Handling and care of loon chicks were done under approval of the US Geological Survey 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 in US Fish and Wildlife Service Scientific Collecting Permit numbers MB030466-3 and MB123047-1 and Wisconsin Department of Natural Resources Scientific Collectors Permit numbers SCP-WCR-104-C, SCP-WCR-115-C, and SCP-WCR-118-C.

Laboratory analyses

Egg and adult loon blood samples were homogenized, digested, and analyzed for trace metals (Al, Ca, Cd, Pb, and Se) by inductively coupled plasma–mass spectrometry on a PerkinElmer ELAN 6000/6100 spectrometer (Frontier SOP FGS-054). Samples for MeHg analysis were analyzed by aqueous phase ethylation, isothermal gas chromatograph (GC) separation, and cold vapor atomic fluorescence spectrometry

detection (Frontier SOP FGS-070). Samples for total Hg analysis were analyzed by $SnCl_2$ reduction, dual gold amalgamation, and cold vapor atomic fluorescence spectrometry detection (Frontier SOP FGS-069). Preparation blanks, certified and standard reference material control samples, and duplicate samples were analyzed concurrently with samples. Certified standard reference control materials used were National Research Council of Canada DOLT-2 (dogfish liver) and National Research Council of Canada DORM-2 (dogfish muscle) for total and MeHg, and National Institute of Standards and Technology (NIST) 1640 (liquid) for Al and Ca. Average recovery rates from the certified values of DOLT-3 and DORM-2 for total Hg were $104.6 \pm 3.1\%$ standard deviation (SD) and $107.5 \pm 2.3\%$. The relative standard deviation for replicate measures of total Hg was $3.1 \pm 2.9\%$. Recovery rates for MeHg for DOLT-3 and DORM-2 averaged $101.1 \pm 3.4\%$ and $95.4 \pm 9.4\%$, respectively. The relative SD for replicate measures of MeHg was $3.2 \pm 2.3\%$. Average recovery rates for Al for certified liquid spikes and NIST 1640 were $90.7 \pm 3.3\%$ and 100%, respectively. Recovery rates for Cd for certified liquid spikes and DORM-2 were $83.9 \pm 0.6\%$ and 107%, respectively. Average recovery rates for Ca for certified liquid spikes and NIST 1640 were $114.4 \pm 16.3\%$ and 116.6%, respectively, whereas the relative SD for replicate sample measures was $2.0 \pm 3.2\%$. Recovery rates for Pb for certified liquid spikes and DORM-2 were $92.3 \pm 1.0\%$ and 89.8%, respectively. Average recovery rates for Se for certified liquid spikes and DORM-2 were $92.1 \pm 12.0\%$ and 111.1%, respectively, whereas the relative SD for replicate samples was $6.3 \pm 8.3\%$.

Total Hg content of loon chick blood, liver, kidney, brain, muscle, feather, carcass (remaining tissue following harvest of organs), and yolk sac samples were determined at the US Environmental Protection Agency's Large Lakes Research Station (Grosse Ile, MI, USA) using either a Milestone DMA-80 or a LECO AMA 254 mercury analyzer. Samples were introduced into the combustion chamber of the instrument, and determinations were made using cold vapor atomic absorption. A PSA analytical automated system, which consists of a GC coupled to an atomic fluorescence detector tuned for Hg, was used for all MeHg analyses as in Bennett et al. [45]. Method blanks, standard reference material (DORM-2 and Quarry Lake Bass [QL25], a US Environmental Protection Agency laboratory standard), and duplicate samples were processed and analyzed concurrently with samples. Average recovery rates from the certified values of DORM-2 and QL25 for total Hg were $103.7 \pm 2.0\%$ and $108.1 \pm 4.8\%$, respectively. Average recovery rates for National Research Council of Canada TORT-2 (lobster hepatopancreas) and DORM-2 for MeHg were $94.3 \pm 5.6\%$ and 88.4%, respectively. The relative SD for replicate measures of total Hg was $2.3 \pm 1.8\%$ and $3.4 \pm 2.0\%$ for MeHg. None of the samples fell below the method detection limit and limit of quantitation. Quality assurance results fell within the criterion established within guidelines of the research station Quality Assurance Plan LLRS-QA-002 [46].

Loon eggs were freeze-dried, after which subsamples were removed and weighed to the nearest microgram on a Sartorious (model M5) microbalance. Fatty acids contained in these samples were quantified in a 3-step process: extraction (using the method of Folch et al. [47]); methylation (using the sulfuric acid in methanol method [48]); and GC analyses following the procedures described in McMeans et al. [49]. Fatty acid methyl esters were quantified using a Hewlett Packard 6890 GC (splitless injection; column = Supelco SP-2560, 100 m × 0.25 mm inner diameter × 0.20-μm-thick film) by comparing peak retention times and areas between the samples and standard curves created using a 37-component fatty acid methyl ester standard (Supelco 47885-U). Fatty acid contents are reported as mass fractions (i.e., μg fatty acid methyl ester/mg dry wt tissue). Estimated extraction efficiencies, obtained by adding a known amount of an internal standard (α-cholestane, a saturated 27-carbon steroid precursor; Sigma C-8003) to each of the tissue samples prior to grinding and extracting averaged $89.3 \pm 21.0\%$ and were used to adjust results. The efficiency of preparing methyl ester derivatives (i.e., fatty acid methyl ester) for GC analyses averaged $100.1 \pm 10.5\%$, and was estimated by adding known amounts of pure standards of 20:2 (cis-11.14-eicosadienoic acid), 20:5n-3 (eicosapentaenoic acid), and 22:6n-3 (docosahexaenoic acid; Sigma E3127, E2011, D2534, respectively) and then quantifying subsequent fatty acid methyl ester yields (using calibration curves prepared with the 37-component fatty acid methyl ester standard, as above) for these 3 compounds.

Statistical analyses

When mass fractions of Se in egg contents were below the detection level of 0.5 μg/g wet weight, values for these eggs were set at one-half the detection level (0.25 μg/g wet wt) in subsequent statistical analyses. We modeled egg Hg, Se, and Ca mass fractions as functions of maternal blood mass fraction, lake pH, lake area, and order-of-laying using linear mixed regression models. Correlation among territories within lakes was addressed by treating residual lake territory effects as normal random variables. A similar analysis was used to assess the contribution of lake pH, chick age, and chick body mass to reduction in chick blood Hg between day of hatch and 30 d to 42 d posthatch. Spearman's correlation analysis was used to explore relations among female blood parameters, egg parameters, and lake characteristics. Differences in tissue Hg content between low- and neutral-pH lakes were determined using Wilcoxon 2-sample tests.

We evaluated models of egg volume that included variables related to body size of maternal loons that produced the eggs, lake characteristics, and levels of Hg in the blood of laying females. Correlation of egg volumes within clutches was addressed by treating residual clutch or lake territory effects as normal random variables. Linear mixed regression models were fitted using maximum likelihood and evaluated using Akaike's Information Criterion corrected for small sample size (AICc) [50]. The AICc is defined as the model –2 log likelihood + the number of estimated parameters (K) × 2 × a small sample correction factor, where the correction factor is $n/(n - K - 1)$ and n = sample size [51]. Support for models was estimated by differences between model-specific AICc values and the AICc value from the model with the smallest or AICc best model. A single such difference was denoted ΔAICc. Models with smaller ΔAICc values indicate greater data support for being the AIC best model. Models with very similar ΔAICc values (e.g., within 2 AICc units) were considered as having approximately equivalent support by study data for being the best model. Akaike weights (w_i), also called model probabilities, provide a measure of the strength of the evidence in favor of each model. A w_i denotes the probability that a given model is the best among the models considered. The w_i is calculated as exp(–ΔAICc/2) and is standardized by dividing by the sum of the exp(–ΔAICc/2) values for all models.

Permutational multivariate analysis of variance (PERMANOVA) was used to evaluate the effect of lake pH on fatty acid

composition of common loon eggs. Fatty acids were square root transformed to down-weight the contributions of quantitatively dominant fatty acids to the similarities between samples. Ordination of samples based on a Euclidian distance resemblance matrix was conducted using the nonmetric multidimensional scaling method of Kruskal [52] and Primer software [53,54]. Because multiple eggs were collected from some lakes, lake was treated as a random variable nested within lake pH category in the analysis; variance was assumed constant among lakes.

We evaluated differences in body size of territorial adult loons on low- and neutral-pH lakes. One missing head length value was estimated using the Primer expectation-maximization algorithm [54], which assumes a multinormal distribution. Variables were ranked, followed by an ordination of samples based on a Euclidian distance resemblance matrix. Then PERMANOVA was used to evaluate pH category, gender, and pH category–gender interaction effects on body size, followed by pairwise tests of size differences with pH category by gender. Because multiple adult loons were measured from some lakes, variance was assumed constant among lakes, and lake was treated as a random variable nested within lake pH category. Contributions of variables to dissimilarity between pH categories were determined using the SIMPER procedure in PRIMER [54]. Analyses not conducted in PRIMER were conducted using SAS Ver 9.2. We used a statistical significance level of 0.05.

RESULTS

Mercury, selenium, and calcium in eggs

The mass fraction of total Hg in 39 common loon eggs collected from 18 lakes varied from 0.17 μg/g to 1.23 μg/g wet weight, and MeHg varied from 0.15 μg/g to 1.06 μg/g wet weight (Table 1). The pH in sampled lakes varied from 5.0 to 8.1. Egg MeHg mass fraction averaged 90.4 ± 0.1% of total Hg content. Both total Hg and MeHg contents in eggs were strongly associated with the mass fractions of maternal blood Hg within a few days of laying ($p \leq 0.0003$) as well as order-of-laying ($p \leq 0.0003$; Table 2). Maternal blood Hg levels in the spring (following laying) varied from 0.29 μg/g to 1.64 μg/g wet weight ($n = 15$). The mass fractions of total Hg in the first egg laid (marked A in Figure 1) averaged approximately 71 ± 4% (standard error) of maternal blood Hg mass fractions, and that of the second egg (marked B) averaged approximately 56 ± 4% of maternal blood Hg level (Figure 1). Mass fractions of Hg and MeHg in the second egg averaged approximately 18 ± 6% and 17 ± 8% less than the first egg laid, respectively. We found weak evidence of negative correlations between pH and both maternal blood Hg and MeHg ($r = –0.42$ and –0.49, $p = 0.12$ and 0.06, respectively; $n = 15$; Spearman rank correlation statistic).

Selenium mass fractions were below the detection level of 0.5 μg/g wet weight in 8 egg content samples. Egg Se mass fraction varied from <0.50 μg/g to 1.06 μg/g wet weight; it was negatively associated with lake pH ($p = 0.02$; Table 2) but was not statistically associated with maternal blood Se levels or egg laying order (Table 2). Egg Se mass fraction was positively correlated with egg total Hg and MeHg mass fractions (Spearman $r = 0.35$ and 0.50, $p = 0.03$ and 0.001, respectively, $n = 31$).

Egg Ca mass fractions varied from 322 μg/g to 940 μg/g wet weight, differed by year of study and egg laying order, were negatively associated with lake surface area, and were marginally associated with lake pH ($p = 0.09$; Table 2). Egg Ca mass fraction was positively correlated with egg Se mass fraction (Spearman $r = 0.46$, $p = 0.004$, $n = 31$).

Egg cadmium and lead mass fractions were below the detection limit, and all eggs sampled but 1 were below the detection limit for aluminum. Consequently, we did not summarize these analytes.

Mercury in chicks

Blood Hg levels in a sample of Wisconsin loon chicks ($n = 42$) ranged from 0.57 μg/g to 3.86 μg/g wet weight at hatch (Table 1). We recaptured and obtained a second blood sample from 22 of the chicks when they were between 30 d and 42 d old. Blood Hg mass fractions declined in growing chicks. The average blood Hg mass fraction in these 22 chicks was 1.5 ± 0.12 μg/g wet weight at hatch, but subsequent blood Hg mass fraction averaged 0.09 ± 0.06 μg/g wet weight, or 5.9 ± 0.5% of the Hg level at hatch. Regression analysis indicated that the proportion of blood Hg mass fraction at 30 d to 42 d old of blood Hg at hatch was negatively associated with lake pH and chick age (Table 2).

Loon chick tissue Hg mass fractions at hatch are summarized in Table 3. Tissue Hg mass fractions were strongly correlated with the mass fraction of Hg in blood and among tissues (Table 4). The mass fraction of Hg in chick blood was negatively associated with lake pH (Spearman $r = –0.68$, $p = 0.004$, $n = 16$). The general pattern of differential Hg content in the internal tissues of chicks at hatch was liver > yolk sac > kidney > muscle > carcass > brain (Table 3). Mass fractions of Hg in brain tissue ranged from 0.23 μg/g to 0.84 μg/g wet weight at hatch. Down feather Hg mass fractions were consistently higher than that of internal tissues, with a range of 6.9 μg/g to 36.6 μg Hg/g wet weight.

The MeHg mass fractions were determined in addition to total Hg in a subset of the tissues. The proportions of total Hg as MeHg in tissues at hatch are summarized in Table 3. Although MeHg comprised approximately 86% of total Hg in blood, the mean percent total Hg as MeHg in most tissues was between 75% and 78%. The exception to this was the yolk sac, where MeHg comprised approximately 57% of total Hg. The

Table 1. Mass fractions of total mercury, methylmercury, selenium, and calcium in common loon maternal blood and fresh eggs, and total mercury in chick blood collected in northern Wisconsin, USA, 2005 to 2006[a]

Parameter	Maternal blood at laying ($n = 15$)	First egg laid ($n = 19$)	Second egg laid ($n = 20$)	Chick blood at hatch ($n = 42$)	Chick blood at 6 wk ($n = 22$)
Total mercury	0.88 ± 0.11 (0.29–1.64)	0.69 ± 0.07 (0.18–1.23)	0.55 ± 0.05 (0.17–0.96)	1.52 ± 0.10 (0.57–3.86)	0.09 ± 0.01 (0.03–0.27)
Methylmercury	0.77 ± 0.10 (0.19–1.71)	0.60 ± 0.06 (0.16–1.06)	0.49 ± 0.05 (0.15–0.93)		
Selenium	2.42 ± 0.21 (1.39–4.55)	0.71 ± 0.04 (0.51–1.00)	0.65 ± 0.04 (0.50–1.06)		
Calcium	101 ± 7 (55–154)	587 ± 34 (322–825)	674 ± 37 (331–940)		

[a]Data are mean ± standard error, with minima and maxima in parentheses (μg/g wet wt).

Table 2. Covariate associations with egg total mercury, methylmercury, selenium, calcium, and chick blood mercury mass fractions collected from northern Wisconsin (USA) lakes during 2005 to 2006[a]

Response variable	Covariate	Estimate (SE)	*t*	*df*	*p*
Egg total mercury	Maternal blood Hg	0.66 (0.06)	11.74	9.9	<0.0001
	Lake pH	0.04 (0.04)	0.83	9.9	0.42
	log(Lake area, km^2)	–0.01 (0.01)	–0.92	10.4	0.38
	Year (ref. = 2006)	–0.01 (0.04)	–0.26	11.2	0.80
	Egg order (ref. = second egg)	0.12 (0.02)	5.80	13.6	<0.0001
Egg methylmercury	Maternal blood MeHg	0.58 (0.11)	5.36	9.9	0.0003
	Lake pH	0.05 (0.08)	0.58	9.9	0.57
	log(Lake area, km^2)	–0.02 (0.02)	–0.93	10.2	0.37
	Year (ref. = 2006)	–0.08 (0.06)	–1.37	10.6	0.20
	Egg order (ref. = second egg)	0.11 (0.02)	4.80	13.3	0.0003
Egg selenium	Maternal blood Se	0.02 (0.05)	0.46	9.8	0.65
	Lake pH	–0.22 (0.08)	–2.80	9.8	0.02
	log(Lake area, km^2)	0.02 (0.03)	0.65	10.1	0.53
	Year (ref. = 2006)	0.04 (0.08)	0.45	10.4	0.66
	Egg order (ref. = second egg)	0.02 (0.03)	0.57	13.1	0.58
Egg calcium	Maternal blood Ca	–0.46 (1.04)	–0.44	10.5	0.67
	Lake pH	67.33 (35.97)	1.87	9.7	0.09
	Lake area (km^2)	–35.68 (12.35)	–2.89	9.8	0.02
	Year (ref. = 2006)	272.10 (57.66)	4.72	11.6	0.0005
	Egg order (ref. = second egg)	–57.79 (19.64)	–2.94	12.6	0.01
Blood Hg mass fraction (30–42 d) as % of blood Hg at hatch	Intercept	5.72 (0.42)	13.66	16	<0.0001
	Lake pH	–3.36 (0.93)	–3.61	16	0.002
	Age	–0.39 (0.18)	–2.17	16	0.046
	Body mass (kg)	1.02 (1.88)	0.54	16	0.595

[a]Estimated using a linear model with random lake term.
SE = standard error; *t* = *t* value; *df* = degree of freedom.

proportion of MeHg generally increased with the mass fractions of tissue total Hg; in kidney tissue, this relation was statistically significant ($r^2 = 0.35$, $p = 0.03$).

Egg fatty acids

Total lipid contents averaged 41.1 ± 0.6% of the dry mass of common loon egg. Total fatty acid mass fraction averaged 311.8 μg fatty acid/mg dry weight (95% confidence limits [CLs], 301.9, 321.7), consisting of 33 fatty acids and dominated by oleic acid (40.5% of total) and palmitic acid (22.8%; Table 5). Five essential fatty acids (α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, linoleic acid, and arachidonic acid) made up 18% of total fatty acids. Overall fatty acid composition did not vary considerably with lake pH category (low vs neutral pH, PERMANOVA, $F_{1,23} = 1.7$, $p = 0.12$), although the essential fatty acid composition was different ($F_{1,23} = 2.9$, $p = 0.01$). This difference was largely attributed to higher average levels of linoleic acid in eggs collected on low-pH lakes ($t = 2.97$, df = 22, $p = 0.007$; Table 6).

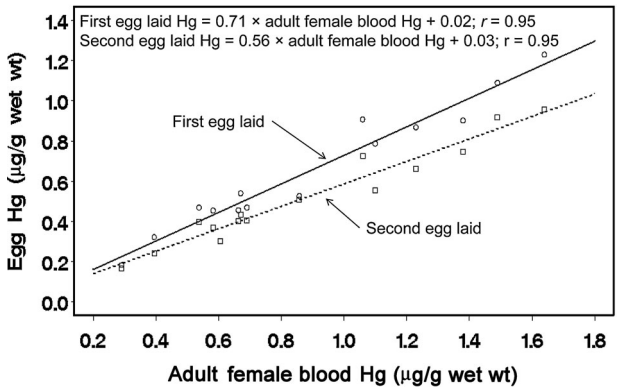

Figure 1. Relationship between mercury mass fractions in the first and second eggs laid per clutch and mass fractions of maternal blood mercury within a few days of laying—northern Wisconsin, 2005 to 2006. First eggs laid are designated with circles, and second eggs laid are designated with squares.

Egg volume

Egg volume of first egg laid averaged 4.7 ± 4.3% (SD) greater than the second egg (minimum –2.9%, maximum 17.9%). The first egg was largest in 23 of 28 (82%) clutches. These differences are reflected in the strong support by data for an egg order effect on egg volume (Table 7). Measured egg

Table 3. Mass fractions of total Hg and ratio of MeHg to total Hg mass fractions in tissues of common loon chicks at hatch collected from lakes in northern Wisconsin, USA, 2005 to 2006

Tissue type	Total Hg mass fraction (mean ± SE, minima–maxima; μg/g wet wt) $n = 16$	Ratio of MeHg to total Hg (mean ± SE) $n = 11$[a]
Brain	0.55 ± 0.07 (0.23–1.39)	0.75 ± 0.01
Carcass	0.77 ± 0.09 (0.35–1.90)	0.75 ± 0.02
Muscle	0.79 ± 0.09 (0.38–1.81)	0.78 ± 0.01
Kidney	1.03 ± 0.12 (0.49–2.61)	0.76 ± 0.01
Yolk sac	1.10 ± 0.11 (0.49–2.36)	0.57 ± 0.03
Liver	1.32 ± 0.14 (0.58–2.93)	0.78 ± 0.01
Blood	1.57 ± 0.19 (0.77–3.86)	0.86 ± 0.02
Down	11.80 ± 1.50 (6.88–31.60)	0.75 ± 0.02

[a]$n = 10$ in the case of liver.
MeHg = methylmercury; SE = Standard error.

Table 4. Pairwise correlation estimates (*r*), with *p* values, for common loon chick blood and tissue Hg mass fractions from northern Wisconsin, USA, 2005 to 2006[a]

	Blood	Liver	Kidney	Brain	Muscle	Yolk sac	Carcass
Liver	0.69, 0.0030						
Kidney	0.66, 0.0051	0.94, <0.0001					
Brain	0.73, 0.0013	0.91, <0.0001	0.94, <0.0001				
Muscle	0.51, 0.0419	0.82, <0.0001	0.91, <0.0001	0.91, <0.0001			
Yolk sac	0.63, 0.0091	0.91, <0.0001	0.94, <0.0001	0.90, <0.0001	0.89, <0.0001		
Carcass	0.68, 0.0041	0.95, <0.0001	0.98, <0.0001	0.95, <0.0001	0.91, <0.0001	0.92, <0.0001	
Down	0.73, 0.0012	0.91, <0.0001	0.92, <0.0001	0.97, <0.0001	0.87, <0.0001	0.88, <0.0001	0.96, <0.0001

[a]Spearman rank correlation statistic; not adjusted for multiple comparisons; $n = 16$ for all comparisons.

volumes, adjusted for order of laying, were strongly correlated ($r = 0.86$, $n = 28$) within nests. Associations with egg volume other than egg order were weak at best. Evidence for association with maternal body length was weak but intriguing, given the relatively small sample size. There was little statistical evidence that maternal Hg burden, body mass, or lake pH were associated with variation in egg volume.

Loon chick mass was strongly associated with egg volume regardless of egg order or year of study (Figure 2). Visual inspection of the panels in Figure 2 suggests that chick mass to egg volume associations among egg order, year, or both do not vary more than trivially.

Adult body size

Body size differed by gender, as expected (PERMANOVA, $F_{1,38} = 7.6, p = 0.01$), and the pH × gender interaction term was also significant (PERMANOVA, $F_{1,38} = 7.1, p = 0.02$; Table 8). Although the overall structural sizes of females on neutral-pH lakes did not differ statistically from those on low-pH lakes ($t = 1.3$, $p = 0.14$), males on neutral-pH lakes were larger on

Table 5. Mean mass fractions of fatty acids of 24 common loon eggs collected in northern Wisconsin, 2007

Fatty acid molecular formula	Common name	Mass fraction (μg/mg dry wt)[a]	% of Total[a]
14:0	Myristic acid	1.62 (1.42, 1.81)	0.52 (0.46, 0.58)
15:0i		0.30 (0.25, 0.34)	0.10 (0.08, 0.11)
15ai		0.10 (0.06, 0.13)	0.03 (0.02, 0.04)
14:1n-5	Myristoleic acid	0.16 (0.13, 0.19)	0.05 (0.04, 0.06)
15:0	Pentadecanoic acid	0.68 (0.60, 0.76)	0.22 (0.19, 0.24)
16:0i		0.17 (0.13, 0.21)	0.05 (0.04, 0.07)
16:0	Palmitic acid	71.08 (68.36, 73.81)	22.79 (22.44, 23.13)
16:1n-7	Palmitoleic acid	10.91 (10.07,11.76)	3.51 (3.25, 3.76)
17:0	Heptadecanoic acid	1.91 (1.72, 2.11)	0.61 (0.55, 0.67)
16:2n-4		0.27 (0.22, 0.31)	0.09 (0.07, 0.10)
16:3n-4		0.14 (0.06, 0.21)	0.04 (0.02, 0.07)
18:0	Stearic acid	20.54 (19.48, 21.60)	6.58 (6.33, 6.84)
18:1n-9t	Elaidic acid	0.39 (0.34, 0.44)	0.13 (0.11, 0.14)
18:1n-9c	Oleic acid	126.17 (121.67, 130.67)	40.50 (39.52, 41.48)
18:1n-7		15.11 (14.24, 15.97)	4.85 (4.60, 5.10)
18:2n-6c	Linoleic acid (LIN)	13.44 (12.16, 14.72)	4.30 (3.92, 4.67)
20:0	Arachidic acid	0.14 (0.10, 0.18)	0.04 (0.03, 0.06)
18:3n-6	γ-Linolenic acid	0.23 (0.20, 0.26)	0.07 (0.07, 0.08
20:1n-9	Eicosenoic acid	0.68 (0.60, 0.77)	0.22 (0.19, 0.25)
20:1n-7		0.06 (0.05, 0.08)	0.02 (0.02, 0.02)
18:3n-3	α-Linolenic acid (ALA)	4.62 (3.99, 5.26)	1.49 (1.28, 1.69)
20:2	cis-11,14-eicosadienoic acid	0.31 (0.28, 0.34)	0.10 (0.09, 0.11)
22:0	Behenic acid	0.22 (0.17, 0.27)	0.07 (0.05, 0.08)
20:3n-6	Homo-γ-linolenic acid	0.24 (0.22, 0.27)	0.08 (0.07, 0.09)
20:3n-3	Eicosatrienoic acid (ETA)	0.16 (0.13, 0.18)	0.05 (0.04, 0.06)
20:4n-6	Arachidonic acid (ARA)	18.95 (17.78, 20.12)	6.07 (5.78, 6.35)
22:2	cis-13,16-docosadienoic acid	0.002 (−0.0, 0.01)	<0.01 (<0.01, <0.01)
24:0	Lignoceric acid	0.02 (−0.0, 0.03)	0.01 (<0.01, 0.01)
20:5n-3	Eicosapentaenoic acid (EPA)	2.62 (2.35, 2.88)	0.84 (0.76, 0.93)
24:1n-9	Nervonic acid	0.06 (0.03, 0.08)	0.02 (0.01, 0.03)
22:4n-6		0.65 (0.60, 0.70)	0.21 (0.19, 0.22)
22:5n-3c	Docosapentaenoic acid (DPA)	3.52 (3.26, 3.77)	1.12 (1.06, 1.19)
22:6n-3	Docosahexaenoic acid (DHA)	16.35 (15.27, 17.43)	5.23 (4.97, 5.48)
∑ω3		27.26 (25.89, 28.63)	8.73 (8.43, 9.03)
∑ω6		33.51 (31.58, 35.45)	10.73 (10.28, 11.18)
∑SAFA		96.77 (93.15, 100.39)	31.01 (30.62, 31.41)
∑MUFA		153.54 (149.02, 158.06)	49.30 (48.55, 50.04)
∑PUFA		61.49 (58.60, 64.39)	19.69 (19.14, 20.24)
Total		311.80 (301.87, 321.74)	

[a]Data are the mean with 95% confidence limits in parentheses.
SAFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.

Table 6. Comparison of essential fatty acid mass fractions in common loon eggs collected from nests on low- and neutral-pH lakes in northern Wisconsin, USA, 2007

Fatty acid		Lake Source[a]			
Molecular formula	Common name	Low pH ($n = 8$)	Neutral pH ($n = 16$)	$t_{df=22}$	p[b]
18:2n-6c	Linoleic acid	15.68 (13.69, 17.66)	12.32 (10.86, 13.77)	2.97	0.007
20:4n-6	Arachidonic acid	19.50 (16.93, 22.07)	18.67 (17.25, 20.10)	0.68	0.505
18:3n-3	α-Linolenic acid	4.80 (3.13, 6.46)	4.54 (3.86, 5.21)	0.39	0.698
20:5n-3	Eicosapentaenoic acid	2.60 (1.99, 3.20)	2.63 (2.31, 2.94)	−0.11	0.915
22:6n-3	Docosahexaenoic acid	17.11 (15.01, 19.21)	15.97 (14.60, 17.34)	1.04	0.311

[a]Data are mean μg/mg dry weight, with 95% confidence limits in parentheses.
[b]Not adjusted for multiple comparisons.
$t = t$ value.

average than those occupying territories on low-pH lakes ($t = 2.6$, $p = 0.001$). A SIMPER analysis indicated that the variables contributing most to this size difference in males were culmen and total head length.

DISCUSSION

Mercury, selenium, and calcium in eggs

Total Hg mass fractions of eggs in the present study reflected the range of mass fractions reported for common loon eggs throughout the Great Lakes states [55,56]. Egg total Hg consisted largely of MeHg (mean = 90.4 ± 0.1%), a finding consistent with that previously reported for common loon eggs collected across Canada (87%; [57]) and North America (98.6 ± 8.2%; [55]).

We observed a very strong relation between egg Hg and maternal blood Hg at time of laying ($r^2 = 0.95$; Figure 1). It should be emphasized that the relation established in the present study applies to female blood Hg at the time of egg laying. Egg laying represents a route of Hg depuration, so the difference in Hg levels between the first and second egg laid likely reflects a decrease in maternal Hg body burden with laying of the first egg. Mercury contents in subsequent blood samples of maternal loons, collected during the chick-rearing season, were usually elevated (up to 3 times higher than spring mass fractions). Increasing adult blood Hg mass fraction during the breeding season likely reflects elevated prey Hg on breeding lakes relative to wintering areas. Foraging occurs on breeding lakes typically for a period of 2 wk to 4 wk prior to egg formation compared with 12 wk to 14 wk of foraging by the time adults were captured during chick-rearing. Timing of sample collections in part contributes to the tighter egg Hg–female blood Hg relation reported in the present study than that provided by Evers et al. ([55]; $r^2 = 0.73$) when the relation to midsummer female blood Hg is described. We suspect that the limited foraging time on breeding lakes prior to egg laying also contributed to the lack of a strong correlation between maternal blood Hg mass fraction at laying and lake pH that is observed later in the summer [11].

Egg Se mass fractions measured in the present study (≤1.06 μg/g wet wt; ≤4.73 μg /g dry wt based on average egg moisture of 77.6%) were well below the established egg Se threshold for adverse effects of 12 μg to 15 μg Se/g dry weight, based on mallard (*Anas platyrhynchos*) egg viability and duckling mortality data [58]. In comparison, Scheuhammer et al. [57] measured egg Se mass fractions that varied from 0.23 μg/g to 1.74 μg/g wet weight with a mean content of 0.6 μg/g wet weight, in common loon eggs collected from various lakes across Canada during 1972 to 1997. The mean Se mass fraction in common loon eggs from Minnesota was 1.91 μg/g dry weight and varied from nondetectable to 3.7 μg/g dry weight [59]. Our observed positive correlation between egg Se and egg Hg mass fractions is consistent with that described for a sample of loon eggs in Canada [57] but differs with the negative correlation observed in common loon eggs analyzed in the Minnesota study [59]. Mercury 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

Table 7. Models used to evaluate the effect of body size of adult females that produced the eggs, lake characteristics, and levels of mercury in the blood of laying females on common loon egg volume in northern Wisconsin, USA ($n = 14$ nests, $n = 2$ eggs per nest)

				Covariate association estimates[b]	
Model[a]	k	ΔAIC_c	w_i	1	2
egg_id (replace with first egg)	3	0	0.31	na	na
egg_id + body (mm)	4	0.66	0.23	0.14 (–0.06, 0.33) mL/mm	na
egg_id + area (ha)	4	1.90	0.12	–0.0003 (–0.0009, 0.0004) mL/ha	na
egg_id + mass (kg)	4	2.50	0.09	8.19 (–19.07, 35.45) mL/kg	na
egg_id + pH	4	2.55	0.09	–1.85 (–8.36, 4.65) mL/pH unit	na
egg_id + blood Hg	4	2.64	0.08	0.004 (–0.011, 0.018) mL/μg/mL	na
egg_id + body (mm) + mass (kg)	5	3.53	0.05	0.19 (–0.11, 0.50) mL/mm	–10.10 (–49.51, 29.31) mL/kg
egg_id + pH + area (ha)	5	5.16	0.02	–0.27 (–8.50, 7.95) mL/pH unit	–0.0003 (–0.0011, 0.0006) mL/ha
Intercept only	2	9.29		na	na

[a]All models include an intercept and a random lake effect. To decrease the probability of entertaining spurious explanatory variables, only models with –2 log likelihood values that differed by ≥1 units from that of the AICc best model were treated as candidates for being the best model [75].
[b]For parameters other than the first egg parameter; the latter parameter was estimated as 6.05 (2.94, 9.16) mL.
na = not available; AICc = Akaike's Information Criterion corrected for small sample size; w_i = Akaike weight.

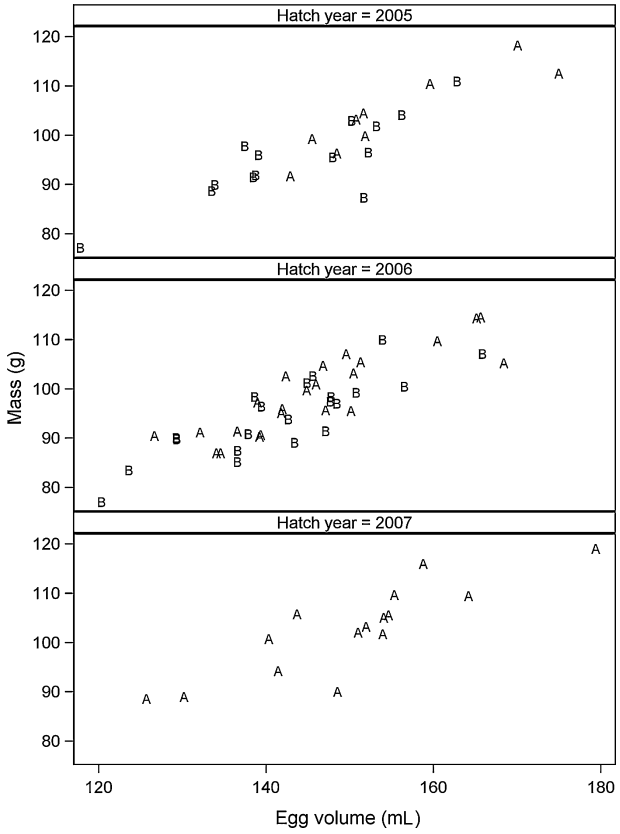

Figure 2. Relationship between common loon chick mass and egg volume for chicks hatched from eggs collected in northern Wisconsin, USA, 2005 to 2007. First eggs laid are designated with an A, and second eggs laid are designated with a B.

protection against Hg-induced toxicity ([60]; see review by Khan and Wang [61]). Khan and Wang [62] have described a mechanism that highlights a potential role of Se for demethylation of MeHg by selenoamino acids, which are prominent in eggs. Synergistic effects of Hg and Se have also been observed in developing embryos [63]. The lack of association between Se and egg laying order was consistent with no significant effect of laying order on Se mass fractions in 2-egg clutches of Audouin's gull (Larus audouinii) eggs [64].

Egg Ca levels did not appear to be limiting with respect to lake pH based on the overwhelming effect of year of collection and the observation that second eggs laid actually had higher mean mass fractions of Ca relative to the first egg laid. We were unable to locate published accounts of common loon egg Ca levels for comparison.

Table 8. Adult common loon measurements (mean ± standard error) by lake source in northern Wisconsin, USA, 2005 to 2006

		Lake source	
Gender	Measurement	Low pH (female $n = 6$; male $n = 6$)	Neutral pH (female $n = 18$; male $n = 18$)
Female	Mass (kg)	3.99 ± 0.08	3.88 ± 0.06
	Culmen (mm)	85.8 ± 1.6	82.5 ± 0.6
	Head (mm)	183.2 ± 2.2	180.2 ± 1.6
	Tarsus (mm)	82.4 ± 1.6	83.7 ± 0.7
	Body length (mm)	782.2 ± 11.8	779.2 ± 6.5
Male	Mass (kg)	4.86 ± 0.12	4.98 ± 0.12
	Culmen (mm)	83.9 ± 1.4	89.1 ± 0.8
	Head (mm)	184.4 ± 1.8	194.0 ± 1.0
	Tarsus (mm)	88.0 ± 1.9	89.3 ± 0.6
	Body length (mm)	810.4 ± 10.3	829.7 ± 3.6

Mercury in chicks

Blood Hg levels in loon chicks at hatch ranged from 0.65 μg/g to 3.86 μg/g wet weight, indicating that exposure in some chicks rivals that of adult birds during the breeding season (range = 0.30–5.32 μg/g wet wt; M. Meyer, unpublished data). Blood Hg levels of maternal loons in this same study area during midsummer varied from 0.34 μg/g to 5.62 μg/g wet weight (mean = 1.73 μg/g). Although initially high at hatch, chick blood Hg mass fractions rapidly declined in growing chicks, likely the result of rapid elimination and growth dilution relative to dietary Hg intake, such that by 6 wk of age, blood Hg levels were approximately 5% of levels at hatch. Fournier et al. [65] documented the importance of feather growth on the toxicokinetics of MeHg, estimating a MeHg elimination half-life of 3 d in loon chicks dosed during feather growth. A 2–compartment elimination model best described the dynamics following completion of feather growth that includes an initial rapid distribution phase with a half-life of 0.9 d, followed by a slow elimination phase with a half-life of 116 d.

Blood Hg mass fractions of chicks at hatch from eggs collected at neutral pH lakes (Table 4) exceeded that which we observed in our laboratory studies of 5-wk-old chicks on a 0.4 μg/g wet weight diet, and blood Hg mass fractions of chicks from eggs collected on low-pH lakes exceeded that of 15-wk-old chicks on a diet containing 0.4 μg Hg/g wet weight [66]. These age-specific blood Hg mass fractions in Hg-dosed chicks were associated with negative physiological effects [18–20]. The pattern of differential Hg content in the tissues of chicks at hatch in the present study was consistent with the pattern observed in Hg-dosed chicks at 35 d and 105 d old [66]. Loon chick brain Hg mass fractions are much lower than those of mallard ducklings (5–8 μg/g wet wt), in which brain lesions were observed [67]. Down feather Hg mass fractions were consistently higher than those of internal tissues and likely represent an important route of Hg elimination in embryos.

In addition to sequestration of MeHg, demethylation provides another mechanism for the developing embryo to deal with MeHg. Our results suggest that the proportion of total Hg as MeHg was lower in tissues than in circulating blood in hatchling loons, indicating demethylation of MeHg. The work of Rutkiewica and Basu [68] indicated that demethylation of MeHg occurred in chicken embryos and hatchlings as part of a MeHgCl egg injection study; they observed a steady increase in Hg mass fractions in tissues throughout embryonic development and presented evidence of demethylation of MeHg in the liver and brains of 19-d-old chicken embryos. We observed a positive relation between the proportion of total Hg as MeHg and total Hg mass fraction, which may reflect an accumulation of MeHg in tissues at a relatively higher rate than inorganic Hg at higher total Hg exposure [69].

We expect that the very low proportion (0.57) of MeHg in the yolk sac may be the result of demethylation in the yolk and/or the result of MeHg more readily taken up by the blood than inorganic Hg. The antioxidant glutathione (GSH) and the antioxidant enzyme GSH peroxidase (GSH-Px) are present in relatively high mass fractions in egg yolk [70], yielding the metabolic capability to demethylate MeHg.

Egg fatty acids

We found no evidence to suggest that overall common loon egg fatty acid composition differed substantially in quality between lake pH categories. However, essential fatty acid composition (largely attributed to linoleic acid) differed with lake pH category. Higher levels of linoleic acid in eggs produced on low-pH lakes likely reflects a greater fraction of dietary lipids of terrestrial origin in the food webs in low-pH lakes [71]. Poultry embryonic viability and hatchability was found to be compromised with linoleic acid deficiency [72]. Deficiency symptoms of linoleic acid in poultry chicks include retarded growth and reduced resistance to disease [73]. However, we found no evidence in previous studies [18,20] to suggest that chicks produced from eggs on neutral-pH lakes exhibited symptoms typically associated with linoleic acid deficiency.

Other essential fatty acids did not differ between lake pH categories, indicating that developing embryos from eggs from low-pH lakes have the complement of fatty acids necessary for proper growth and function. Fatty acid deficiency is apparently not a factor contributing to differences in chick quality (i.e., lower growth rates, behavioral differences, and evidence of lymphoid depletion in low-pH lake chicks relative to chicks from neutral-pH lakes) observed in previous studies.

Egg volume

The evidence shown in the present study for an association between maternal body size (length) and egg volume, although weak, is congruent with prevailing hypotheses. Larger egg volume in turn relates to higher quality chicks (based on the literature provided in the *Introduction*). Indeed, loon chick mass in the present study was strongly correlated with egg volume (Figure 2). We originally speculated that maternal body size may account for the size differences in chicks produced on low- versus neutral-pH lakes. However, our findings that body size of maternal loons did not vary statistically with lake pH do not support this hypothesis. Sample size was such that the probability of finding relatively weak effects was undoubtedly low.

Adult body size

Although body size of maternal loons on neutral-pH lakes did not differ significantly from those on low-pH lakes, males on neutral-pH lakes were larger (culmen length, head length, body length, body mass) than those on low-pH lakes and may have genetic implications for differences in lake-source–related chick quality. Morphological traits are moderately to highly heritable [74] depending on interactions between genotype and favorability of environmental conditions for offspring development. Although previous studies have noted that availability of food resources contributes to observed differences in chick survival (prefledging survival [2,25], juvenile [fledging to 2 yr] survival [25]) and chick mass [25], clearly in ovo effects exist with persistent consequences on chick development (including growth) that are independent of food availability [18]. Unfortunately, no direct evidence is available linking quality of breeding in common loons and implications for the growth and survival of their progeny.

In pondering the question of why loons attempt to breed on small, low-pH lakes, Piper et al. [25] hypothesized "that small lakes are marginal habitat used by inferior individuals and/or those in subprime condition." Our results support this notion: in the case of males, loons holding territories on inferior, low-pH lakes are structurally smaller than those holding preferred territories on neutral-pH lakes.

The 2 factors that were statistically related to lake pH in the field, and thus the lake source effect measured in the laboratory, were adult male body size and chick Hg exposure. We recommend that future research into the effects of contaminants on common loon chick quality and survival include evaluation of the inheritance of ecologically important traits, especially where the effects of lake characteristics (e.g., pH), parental stature, and contaminant sources are potentially confounding. Also, researchers should consider the value of obtaining structural measures of loons in addition to body mass, and should recognize the value of obtaining samples and measures of breeding adults at the time of egg laying when relating adult and egg and juvenile contaminant levels.

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Data availability—Data are available on request from the authors. Address correspondence to the first author (kkenow@usgs.gov).

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