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Methylmercury is the Predominant Form of Mercury in Bird Eggs: A Synthesis

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ABSTRACT: Bird eggs are commonly used in mercury monitoring programs to assess methylmercury contamination and toxicity to birds. However, only 6% of >200 studies investigating mercury in bird eggs have actually measured methylmercury concentrations in eggs. Instead, studies typically measure total mercury in eggs (both organic and inorganic forms of mercury), with the explicit assumption that total mercury concentrations in eggs are a reliable proxy for methylmercury concentrations in eggs. This assumption is rarely tested, but has important implications for assessing risk of mercury to birds. We conducted a detailed assessment of this assumption by (1) collecting original data to examine the relationship between total and methylmercury in eggs of two species, and (2) reviewing the published literature on mercury concentrations in bird eggs to examine whether the percentage of total mercury in the methylmercury form differed among species. Within American avocets (*Recurvirostra americana*) and Forster's terns (*Sterna forsteri*), methylmercury concentrations were highly correlated (R^2 = 0.99) with total mercury concentrations in individual eggs (range: 0.03−7.33 μg/g fww), and the regression slope (log scale) was not different from one (m = 0.992). The mean percentage of total mercury in the methylmercury form in eggs was 97% for American avocets (n = 30 eggs), 96% for Forster's terns (n = 30 eggs), and 96% among all 22 species of birds (n = 30 estimates of species means). The percentage of total mercury in the methylmercury form ranged from 63% to 116% among individual eggs and 82% to 111% among species means, but this variation was not related to total mercury concentrations in eggs, foraging guild, nor to a species life history strategy as characterized along the precocial to altricial spectrum. Our results support the use of total mercury concentrations to estimate methylmercury concentrations in bird eggs.

■ INTRODUCTION

Methylmercury is a global pollutant that biomagnifies primarily through aquatic food chains and is toxic to humans and wildlife.[1] As top predators in many aquatic habitats, birds are often among the taxa most vulnerable to environmentally relevant levels of methylmercury exposure[2] and therefore are the focus of many mercury monitoring programs.[3−7] Impaired reproduction is the most common toxicity end point for methylmercury exposure in birds,[2,8] and consequently eggs are the focus of many studies examining methylmercury bioaccumulation and toxicity in birds.

It is generally assumed that most of the mercury in bird eggs is in the more bioaccumulative and toxic form—methylmercury. Yet few studies have actually measured methylmercury concentrations in eggs. Instead, studies typically measure total mercury concentrations (both organic and inorganic forms of mercury) in eggs because it is much less expensive and easier to determine analytically. These methods often allow studies with limited budgets to significantly increase their sample sizes. The explicit assumption is that total mercury concentrations in eggs are a reliable estimate of methylmercury concentrations in eggs. This assumption is rarely tested but has important implications for assessing the risk of mercury to birds, since it is the methylmercury form of the contaminant that poses the greatest risk to embryo toxicity.[1,2]

For total mercury concentrations to be a reliable proxy for methylmercury concentrations in bird eggs, several criteria should be met. First, the majority of mercury in bird eggs should be in the methylmercury form. This criterion has been the focus for the few studies that have examined methylmercury in bird eggs, and generally has been supported (Table 1). Second, methylmercury concentrations in eggs should be highly correlated with total mercury concentrations in eggs. This criterion has been tested only twice prior to this paper, and was supported just once.[9,10] Third, the percentage of total mercury in the methylmercury form should not change as a function of total mercury concentrations in eggs. There are several mechanisms that might cause the relationship between the percentage of total mercury in the methylmercury form to

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Table 1. Synthesis of All the Published Literature on the Percentage of Total Mercury (THg) in the Methylmercury (MeHg) Form in Bird Eggs

species	latin	foraging guild[a]	precocial to altricial spectrum (ranked 1−8)[b]	sample size for MeHg in eggs	mean % of THg in MeHg form in eggs	sample size for THg in eggs	mean THg in eggs (μg/g dw)	site	year	citation
American avocet	*Recurvirostra americana*	omnivore	precocial-2 (2)	30	97.1	30	1.48	San Francisco Bay, CA	2011	Ackerman et al. (this paper)
Forster's tern	*Sterna forsteri*	piscivore	semi-precocial (5)	30	95.9	30	8.56	San Francisco Bay, CA	2011	Ackerman et al. (this paper)
American kestrel[c,d,e]	*Falco sparverius*	insectivore	semi-altricial-1 (6)	1	98.2	1	33.36	lab cages	2000−2004	Nichols et al.;[33] J. Nichols and R. Bennett, Pers. comm.
American kestrel[c,d,f]	*Falco sparverius*	insectivore	semi-altricial-1 (6)	6	97.1	6	72.28	lab cages	2000−2004	Nichols et al.;[33] J. Nichols and R. Bennett, Pers. comm.
Leach's storm-petrel	*Oceanodroma leucorhoa*	carnivore	semi-precocial (5)	5	88.5	5	1.17	Machias Seal Island, New Brunswick, Canada	2006	Bond and Diamond[10]
cliff swallow[g]	*Petrochelidon pyrrhonota*	insectivore	altricial (8)	7	110.8	7	0.26	Cache Creek, CA	1997	Hothem et al.[35]
California clapper rails	*Rallus longirostris obsoletus*	crustacivore/molluscivore	precocial-4 (4)	17	94.7	17	3.13	San Francisco Bay, CA	1991−1999	Schwarzbach et al.[39]
common loon[h]	*Gavia immer*	piscivore	precocial-4 (4)	20	98.6	577	2.89	lakes in North America	1988−2001	Evers et al.[4]
common loon[h,j]	*Gavia immer*	piscivore	precocial-4 (4)	24	87.0	125	2.30	lakes in Canada	1972−1997	Scheuhammer et al.[9]
Forster's tern	*Sterna forsteri*	piscivore/insectivore	semi-precocial (5)	6	94.0	6	7.30	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
Caspian tern	*Hydroprogne caspia*	piscivore	semi-precocial (5)	3	101.3	3	4.66	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
double-crested cormorant	*Phalacrocorax auritus*	piscivore	altricial (8)	6	103.0	6	2.70	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
black-necked stilt	*Himantopus mexicanus*	insectivore	precocial-2 (2)	3	93.0	3	1.73	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
great egret	*Ardea albus*	carnivore/crustacivore	semi-altricial-1 (6)	3	90.3	3	1.60	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
Brandt's cormorant	*Phalacrocorax penicillatus*	piscivore	altricial (8)	3	109.2	3	1.58	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
American avocet	*Recurvirostra americana*	omnivore	precocial-2 (2)	3	111.0	3	1.14	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
snowy egret	*Egretta thula*	crustacivore/carnivore	semi-altricial-1 (6)	3	100.6	3	0.94	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
American bittern	*Botaurus lentiginosus*	carnivore/insectivore/crustacivore	semi-altricial-1 (6)	2	97.0	2	0.82	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
black-crowned night heron	*Nycticorax nycticorax*	piscivore/crustacivore	semi-altricial-1 (6)	3	95.7	3	0.80	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
great blue heron	*Ardea herodias*	piscivore	semi-altricial-1 (6)	1	81.5	1	0.67	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
western gull	*Larus occidentalis*	carnivore	semi-precocial (5)	3	109.8	3	0.28	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
mallard	*Anas platyrhynchos*	granivore	precocial-2 (2)	3	108.7	3	0.15	San Francisco Bay, CA	2000−2001	Schwarzbach and Adelsbach[30]
great egret	*Ardea albus*	carnivore/crustacivore	semi-altricial-1 (6)	20	84.9	20	1.64	Everglades, FL	1999	Rumbold et al.[34]
northern gannet[k]	*Morus bassanus*	piscivore	altricial (8)	10	91.1	10	2.04	coast of Norway	1972	Fimreite et al.[31]

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Table 1. continued

species	latin	foraging guild[a]	precocial to altricial spectrum (ranked 1−8)[b]	sample size for MeHg in eggs	mean % of THg in MeHg form in eggs	sample size for THg in eggs	mean THg in eggs (μg/g dw)	site	year	citation
common murre[k]	*Uria aalge*	piscivore/crustacivore	precocial-2 (2)	9	97.6	9	0.52	coast of Norway	1972	Fimreite et al.[31]
herring gull[k]	*Larus argentatus*	omnivore	semi-precocial (5)	13	91.9	13	1.04	coast of Norway	1972	Fimreite et al.[31]
domestic chicken[d,l]	*Gallus gallus domesticus*	granivore	precocial-3 (3)	2	95.0	2	24.24	poultry cages	approximately 1980	Cappon and Smith[14]
domestic chicken[d,e,l,m]	*Gallus gallus domesticus*	granivore	precocial-3 (3)	80	90.3	80	1.24	poultry cages	1969	Kimimäe et al.[12]
domestic chicken[d,f,l,m]	*Gallus gallus domesticus*	granivore	precocial-3 (3)	72	89.1	72	2.12	poultry cages	1969	Kimimäe et al.[12]
domestic chicken[k,l]	*Gallus gallus domesticus*	granivore	precocial-3 (3)	17	88.0	na	na	poultry cages	1967−1968	Westöö[32]
total				*388*	*96.4*					

[a]Species foraging guilds were classified following De Graaf et al.[37] We used the foraging guild associated with the breeding season when these data were available, otherwise we used the year-round foraging guild. [b]Species ranked from 1 to 8 along the precocial to altricial spectrum following Nice,[36] Carey et al.,[17] Sotherland and Rahn,[18] and Starck and Ricklefs.[19] Precocial-1 = 1; precocial-2 = 2; precocial-3 = 3; precocial-4 = 4; semi-precocial = 5; semi-altricial-1 =6; semi-altricial-2 = 7; altricial = 8. [c]Total mercury concentrations in eggs was not reported in the publication, but was related through a personal communication with J. Nichols and R. Bennett. We separated the data into the two dosing groups. [d]Data from a study that fed breeding females a diet dosed with methylmercury. [e]Low dose group. [f]High dose group. [g]Data based on composite samples of 3−4 eggs per site; not individual eggs. [h]Note that the sample sizes differ between the estimates for percent of total mercury in the methylmercury form in eggs and total mercury concentration in eggs because both values were not provided for just the subset of eggs that were analyzed for methylmercury. [j]The percent of total mercury in the methylmercury form in eggs was adjusted by Scheuhammer et al.[9] from 75% methylmercury in eggs to 87% using an 86% percent recovery of methylmercury for standard reference material. [k]Only range of data was provided; no other variance data available. [l]Data from albumen only; not whole egg. [m]Data were averaged for different types of mercury administered and for different durations of dosing. We separated the data into the two dosing groups.

change with total mercury concentrations in eggs. For example, birds have the ability to demethylate methylmercury in their livers when exposure levels exceed a threshold value, and there are differences among species in demethylation abilities.[11] Therefore, depending on the proximate mechanisms for maternal transfer of mercury into eggs, it is possible that the percentage of mercury in the methylmercury form in eggs could decline with increasing total mercury concentrations. Fourth, in order for studies to be comparable among species, the percentage of mercury in the methylmercury form in eggs should not vary among species or species groups, such as foraging guild or life history strategy. For example, mercury is more prevalent in egg albumen than in egg yolk, and the percentage of total mercury in the methylmercury form is much greater in egg albumen than in egg yolk.[10,12−16] Egg yolks of altricial species tend to be smaller in relation to egg size and have higher water content and lower energy than egg yolks of precocial species.[17−19] Therefore, it is possible that eggs of altricial species might have a higher percentage of total mercury in the methylmercury form than more precocial species, due to the relative composition of eggs among species and the high affinity of methylmercury to egg albumen.

Herein, we conducted a detailed assessment of the validity of using total mercury concentrations as a reliable proxy for methylmercury concentrations in bird eggs. To do so, we collected original data to examine egg mercury relationships within individual species, and also summarized the published literature on methylmercury concentrations in bird eggs to assess relationships among species. We examined both methylmercury and total mercury concentrations in eggs of two species that are known to have relatively high (Forster's terns, *Sterna forsteri*) and more moderate (American avocets, *Recurvirostra americana*) exposures to mercury,[20,21] which provided us the ability to test the relationship between methylmercury and total mercury concentrations in eggs over a wide range of exposure levels. These two species also represented different trophic levels and foraging guilds, with Forster's tern's diet consisting mainly of fish[22] and American avocet's diet consisting of aquatic invertebrates.[23] We then synthesized all the peer-reviewed literature on methylmercury concentrations in bird eggs to examine whether the percentage of total mercury in the methylmercury form differed among species, and particularly if it varied among foraging guilds or increased along the precocial to altricial spectrum among species.

EXPERIMENTAL SECTION

Detailed Study of Methylmercury and Total Mercury in Bird Eggs. We conducted a detailed assessment of methylmercury concentrations in eggs of Forster's terns and American avocets nesting in San Francisco Bay, CA (37.4° N, 122.0° W) during 2011. We collected eggs at several wetlands (A1, A2W, A7, A8, E7, R1, SF2, and New Chicago Marsh) where mercury concentrations in birds are known to vary substantially.[20,21,24,25] We entered colonies weekly, marked each new nest, and determined incubation stage via egg floatation.[26] We randomly collected one egg each from 30 Forster's tern and 30 American avocet clutches. We stored eggs in a refrigerator until dissection (within 19 ± 10 days [mean ± SD]). We measured length and breadth of each egg to the nearest 0.01 mm using digital calipers (Fowler, Newton, MA) and measured total egg weight to the nearest 0.01 g on a digital balance (Ohaus Adventurer Pro, Ohaus Corporation, Pine Brook, NJ). We cut an ∼15-mm diameter hole in the top of each egg using clean, stainless steel scissors, and removed the embryo and any remaining contents into a sterile 30−60 mL jar with stainless steel forceps. We measured total egg content weight with a digital balance to the nearest 0.01 g. Egg contents were then stored frozen at −20 °C until mercury determination.

We processed and analyzed egg samples for total mercury (THg) and methylmercury (MeHg). We dried the entire egg contents at 50 °C for 48 h until completely dried and reweighed egg contents to determine moisture content. We then ground the dried egg contents to a powder in a Wiley mill and mortar and pestle. We subsampled each dried and homogenized egg twice for separate determination of THg and MeHg concentrations. We determined THg concentrations at the U.S. Geological Survey, Davis Field Station Mercury Lab on a Milestone DMA-80 Direct Mercury Analyzer (Milestone, Monroe, CT) following Environmental Protection Agency Method 7473.[27] We determined MeHg concentrations at Battelle Marine Sciences Laboratory (Sequim, WA) using cold vapor atomic fluorescence following Environmental Protection Agency Method 1630.[28] Quality assurance measures included analysis of two certified reference materials (either dogfish muscle tissue [DORM-3], dogfish liver [DOLT-3 or DOLT-4], or lobster hepatopancreas [TORT-2] by the National Research Council of Canada, Ottawa, Canada), two system and method blanks, three continuing calibration verifications, and two duplicates per batch. Recoveries (mean ± SE) for certified reference materials were 100% ± 1.3% (n = 18) for THg in eggs and 98 ± 3.9% (n = 3) for MeHg in eggs, and for calibration verifications were 99 ± 1.6% (n = 27) for THg in eggs and 100 ± 2.5% (n = 3) for MeHg in eggs. Absolute relative percent difference for duplicates averaged 6.3 ± 1.2% (n = 27) for THg in eggs and 3.7 ± 0.3% (n = 3) for MeHg in eggs.

Because eggs can lose a substantial amount of weight from the time of laying due to respiration and moisture loss, THg and MeHg concentrations in eggs were determined on a dry weight basis and then converted into a fresh wet weight egg concentration. To do so, egg Hg concentrations on a dry weight basis were first converted to egg Hg concentrations on a wet weight basis using an individual egg's specific moisture content. Then, egg Hg concentrations on a wet weight basis were converted to egg Hg concentrations on a fresh wet weight basis by dividing the total wet weight of the egg at dissection by the predicted fresh wet weight of the egg at laying (see below) and multiplying that value by the egg Hg concentration on a wet weight basis at dissection. The fresh wet weight of the egg at laying was estimated by multiplying an individual egg's volume (estimated following Hoyt[29]) by the density of a typical freshly laid egg. We used an egg volume coefficient (K_v) of 0.487 for Forster's terns and 0.467 for American avocets, and an egg density of 1.025 (g/mL) for both Forster's terns and American avocets (J.T. Ackerman, unpublished data).

Review of Published Literature on Methylmercury and Total Mercury in Bird Eggs. We conducted a thorough literature review of all peer-reviewed journal articles assessing Hg concentrations in bird eggs, and then reviewed the articles in detail to determine whether they specifically examined MeHg concentrations in eggs. We also included an unpublished, peer-reviewed report by Schwarzbach and Adelsbach.[30]

We summarized all literature by calculating, or recording if provided, the mean and standard deviation of the percentage of THg in the MeHg form in eggs by species (Table 1). For six species, standard deviations could not be estimated based on the published results. These included four species estimates (northern gannet, common guillemot, herring gull, and domestic chicken) where only the range of percent MeHg in eggs was provided,[31,32] two species (great blue heron and American kestrel) where only one egg was sampled and therefore there was no variance,[30,33] and another species (common loon) where no variance data was included.[9] In most studies, the percentage of THg in the MeHg form was calculated by dividing MeHg by THg concentrations that were determined separately. For Cappon and Smith[14] and Westöö,[32] THg concentrations were calculated by summing inorganic and organic Hg concentrations.

To examine whether the percentage of THg in the MeHg form was related to THg concentrations in eggs, we also summarized the mean and standard deviations of THg concentrations in eggs for each study (Table 1). Although it is preferred to report egg Hg concentrations on a fresh wet weight basis, most studies did not and it was not possible to convert the reported egg THg concentrations into a fresh wet weight basis because individual egg morphometric data was not reported. Therefore, to provide a common reporting standard for study comparison purposes, we converted egg THg concentrations (μg/g) into a dry weight basis if they were not already reported in dry weight. Because some studies did not report moisture content,[12,14,31,33,34] we converted egg THg concentrations on a wet weight basis into dry weight using an average moisture content of 75% (mean moisture content of eggs in Table 1).

We were able to estimate standard deviations for egg THg concentrations (μg/g dry weight) in all but five species. For three species (northern gannet, common guillemot, and herring gull) only the range of THg concentrations was provided.[31] For two species (great blue heron and American kestrel) only one egg was sampled and therefore there was no variance.[30,33] We excluded the study by Westöö[32] since only a range of THg concentrations was reported.

Where possible, we estimated mean THg concentrations from the same eggs that were used to estimate the percentage of THg in the MeHg form. However, when these data were not provided, overall mean THg concentrations in eggs were substituted (great egrets[34] and common loons[4,9]). This discrepancy is noted in Table 1 by comparing sample sizes for the percentage of THg in the MeHg form and for THg concentrations. For one species (great egrets), the mean and standard deviation of THg concentrations in eggs was estimated based on the weighted average of site-level means because overall means were not provided.[34] For another species (cliff swallows), values are based on composite samples of 3–4 eggs per site, rather than individual eggs.[35]

We assessed whether the percentage of THg in the MeHg form was related to species life history strategy. To do so, we defined a species life history strategy on a continuous scale from one to eight along the precocial to altricial spectrum according to hatchling maturity (Table 1) developed by Nice[36] and summarized by Carey et al.,[17] Sotherland and Rahn,[18] and Starck and Ricklefs.[19] We also tested whether the percentage of THg in the MeHg form in bird eggs varied among species according to their foraging guild. We categorized each species foraging guild according to De Graaf et al.[37] We used the foraging guild associated with the breeding season when these data were available, otherwise we used the year-round diet.[37] We also used the primary foraging guild for statistical analyses when multiple foraging guilds were listed for a species.[37]

Statistical Analysis. We used multiple linear regression to examine the relationship between MeHg concentrations (μg/g fresh wet weight, hereafter fww) and THg concentrations (μg/g fww) in individual bird eggs, with species (American avocet or Forster's tern) and species × egg THg concentration interaction as factors (JMP version 10.0.0). We used the likelihood ratio test to determine if adding the species and species × egg THg concentration interaction to the reduced model substantially improved model fit. For the best model, we then used a *t*-test to determine whether the slope of the relationship differed from a value of one. Similarly, we used multiple linear regression to examine the relationship between the percentage of THg in the MeHg form and THg concentrations (μg/g fww) in bird eggs, with species and species × egg THg concentration interaction as factors. We similarly used the likelihood ratio test to determine model fit.

We used the summarized data from our literature review to further examine whether the percentage of THg in the MeHg form differed among species according to their overall THg concentration, foraging guild, or life history strategy. We used linear regression to examine the relationship between the mean percentages of THg in the MeHg form and mean THg concentrations (μg/g dry weight, hereafter dw) in bird eggs. We also used linear regression to examine whether the percentage of THg in the MeHg form in bird eggs increased along the precocial to altricial spectrum among species (1–8). We used analysis of variance (ANOVA) to test whether the proportion of THg in the MeHg form in bird eggs differed among foraging guilds.

Because sample size and variation differed for each of the estimates of the percentage of THg in the MeHg form, we repeated the above three analyses using weighted regression or weighted ANOVA which placed more emphasis on the estimates that had more precision. To do so, each data point was weighted by the inverse of the squared standard error of the estimate. For these weighted analyses, we excluded six species estimates where no variance data were available (see above) and five additional species estimates which were either second, third, or fourth estimates for the same species. Including only a single estimate for each species (we selected the estimate with the largest sample size) confirmed that these duplicated species estimates did not influence the results. We tested for heteroscedasticity in the data using the Breusch–Pagan test.[38] We $\log_e$-transformed egg THg and MeHg concentrations for all statistical analyses, and presented raw data in graphs on a $\log_{10}$ scale.

RESULTS

The percentage of THg in the MeHg form in eggs was 97.1% (93.6–100.6%) for American avocets (n = 30) and 95.9% (92.6–99.3%) for Forster's terns (n = 30; mean and 95% CI). THg concentrations in eggs ranged from 0.03 to 1.99 μg/g fww in American avocets and 0.56 to 7.33 μg/g fww in Forster's terns, representing a 244-fold difference in the range of observed egg THg concentrations. Individual egg MeHg concentrations (μg/g fww) were highly correlated with THg concentrations (μg/g fww) in American avocets and Forster's terns (R^2 = 0.99, $F_{1,58}$ = 9634.71, p < 0.0001; Figure 1). Using the likelihood ratio test, we found that including species (n =

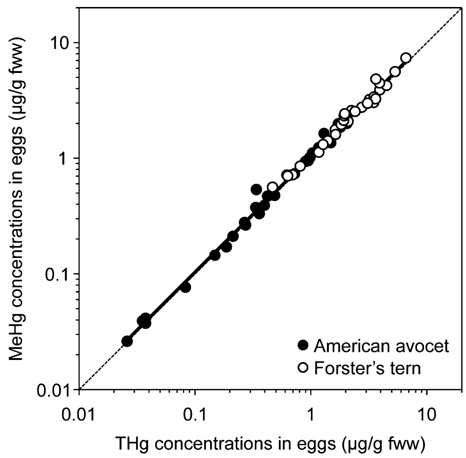

Figure 1. Methylmercury concentrations (MeHg; μg/g fresh wet weight [fww]) were highly correlated with total mercury concentrations (THg; μg/g fww) in individual eggs of American avocets (n = 30; closed symbols) and Forster's terns (n = 30; open symbols) from San Francisco Bay, California. The stippled line indicates a one-to-one relationship (slope of 1.0). The linear regression equation describing the significant relationship was $\log_e$ (MeHg) = −0.041 + 0.992($\log_e$ THg).

60, χ^2 = 0.01, p = 0.94) and species × egg THg concentration interaction (n = 60, χ^2 = 0.79, p = 0.67) did not improve the reduced model's fit describing the relationship between MeHg and THg concentrations in individual eggs. Importantly, the slope estimate was 0.992 (95% CI: 0.97−1.01) and was not different from 1.00 (t = 0.75, df = 58, p = 0.46), indicating a one-to-one relationship between egg MeHg concentrations and THg concentrations.

The percentage of THg in the MeHg form in eggs ranged from 63.4% to 109.8% in American avocets and 75.7% to 115.7% in Forster's terns. The percentage of THg in the MeHg form was not related to THg concentrations (μg/g fww) in individual eggs of American avocets and Forster's terns (R^2 = 0.01, $F_{1,58}$ = 0.57, p = 0.45; Figure 2). Using the likelihood ratio test, we found that including species (n = 60, χ^2 = 0.01, p = 0.99) and species × egg THg concentration interaction (n = 60, χ^2 = 0.98, p = 0.61) also did not improve the reduced model's fit to the data. The variability of the residuals from the linear

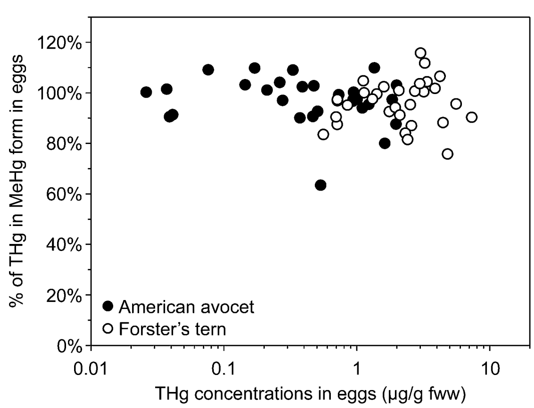

Figure 2. Within species, the percentage of total mercury (THg) in the methylmercury (MeHg) form was not correlated with total mercury concentrations (μg/g fresh wet weight [fww]) in individual eggs of American avocets (n = 30; closed symbols) and Forster's terns (n = 30; open symbols) from San Francisco Bay, California.

model was not dependent on THg concentrations (μg/g fww) in eggs (test for heteroscedasticity: n = 60, χ^2 = 0.24, p = 0.62), indicating that the variability in the percentage of THg in the MeHg form was similar at lower and higher THg concentrations in eggs.

Of 203 peer-reviewed articles that examined Hg concentrations in bird eggs as of October 2012, only 6% of studies (11 journal articles and one report) examined MeHg concentrations in eggs and had a median sample size of just 6 eggs (Table 1). Among all estimates for the 22 species studied, the mean percentage of THg in the MeHg form was 96.4 ± 7.8% (mean ± SD) and single-species means ranged from 82% to 111% (n = 30 estimates of species means). Similar to our results within species, the mean percentage of THg in the MeHg form in eggs was not correlated with mean THg concentrations (μg/g dw) in bird eggs among species (R^2 = 0.05, n = 29, $F_{1,27}$ = 1.46, p = 0.24; Figure 3), nor was it

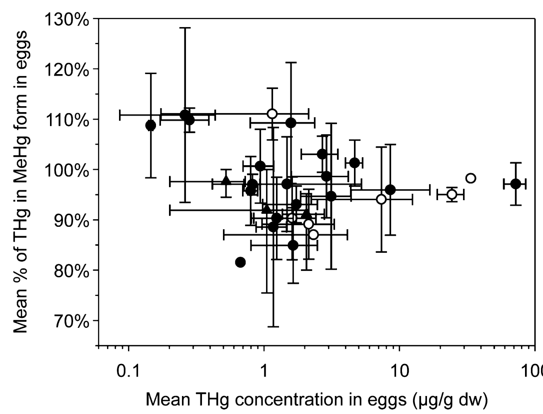

Figure 3. Among 22 species of birds, the mean percentage of total mercury (THg) in the methylmercury (MeHg) form in eggs was not correlated with mean total mercury concentrations (μg/g dry weight [dw]) in eggs (n = 29). Error bars represent the standard deviation for each species except for three species where the error bars represent the range of the data (these three data points are noted with triangles instead of circles). Open symbols indicate (second or third) data points for species with multiple estimates. Data points represented by triangle symbols or open symbols and those without variance were omitted from a secondary analysis which used weighted regression that placed more emphasis on the estimates which had more precision.

correlated within a reduced data set using a weighted regression and no duplicated species estimates (R^2 = 0.04, n = 18, $F_{1,16}$ = 0.67, p = 0.43). The variability of the residuals from the linear model tended to decrease with THg concentrations (μg/g dw) in eggs (test for heteroscedasticity: n = 29, χ^2 = 4.03, p = 0.04 [larger data set]; n = 18, χ^2 = 0.36, p = 0.55 [reduced data set]), indicating that the variability in the percentage of THg in the MeHg form tended to be greater at lower THg concentrations in eggs.

The mean percentage of THg in the MeHg form in eggs was not related to species ranking along the precocial to altricial spectrum when using either the larger data set (R^2 = 0.01, n = 30, $F_{1,28}$ = 0.22, p = 0.64; Figure 4) nor when using a weighted regression and no duplicated species estimates in a reduced data set (R^2 = 0.14, n = 18, $F_{1,16}$ = 2.66, p = 0.12). Additionally, the mean percentage of THg in the MeHg form in eggs did not vary among foraging guilds when using either the larger data set (n = 30, $F_{5,24}$ = 0.41, p = 0.84; Figure 5) nor when using a

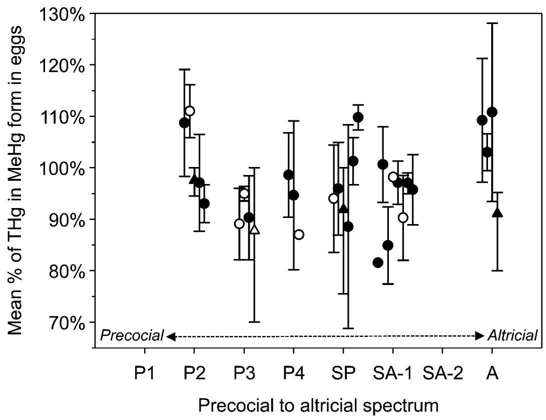

Figure 4. Among 22 species of birds, the mean percentage of total mercury (THg) in the methylmercury (MeHg) form in eggs was not related to a species life history strategy, defined on a continuous scale from one to eight along the precocial to altricial spectrum according to hatchling maturity (n = 30). P1 = Precocial-1; P2 = precocial-2; P3 = precocial-3; P4 = precocial-4; SP = semi-precocial; SA-1 = semi-altricial-1; SA-2 = semi-altricial-2; A = altricial (following Nice[36]). Overlapping data points in each group are offset in the figure for clarity. There were no data for groups P1 or SA-2. Error bars represent the standard deviation for each species except for four species where the error bars represent the range of the data (these four data points are noted with triangles instead of circles). Open symbols indicate (second, third, or fourth) data points for species with multiple estimates. Data points represented by triangle symbols or open symbols and those without variance were omitted from a secondary analysis which used weighted regression that placed more emphasis on the estimates that had more precision.

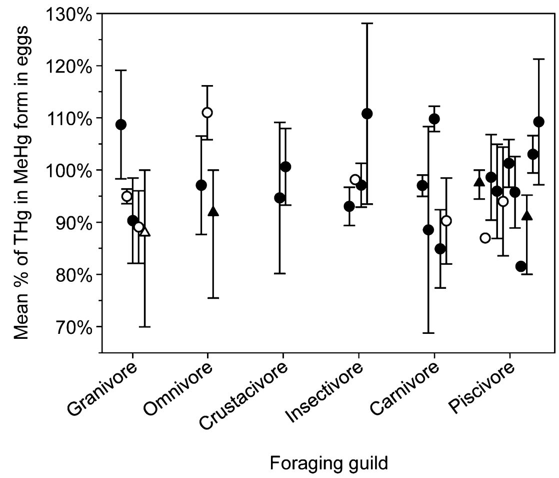

Figure 5. Among 22 species of birds, the mean percentage of total mercury (THg) in the methylmercury (MeHg) form in eggs was not related to a species primary foraging guild (n = 30). Species foraging guilds were classified following De Graaf et al.[37] Overlapping data points in each group are offset in the figure for clarity. Error bars represent the standard deviation for each species except for four species where the error bars represent the range of the data (these four data points are noted with triangles instead of circles). Open symbols indicate (second, third, or fourth) data points for species with multiple estimates. Data points represented by triangle symbols or open symbols and those without variance were omitted from a secondary analysis which used weighted regression that placed more emphasis on the estimates that had more precision.

weighted ANOVA and no duplicated species estimates in a reduced data set (n = 18, $F_{5,12}$ = 0.89, p = 0.52).

DISCUSSION

Bird eggs are commonly used in Hg monitoring programs to examine the spatial and temporal extent of MeHg contamination in the environment and the potential for MeHg toxicity to birds. Yet, only 6% of more than 200 studies that investigated Hg concentrations in bird eggs actually measured MeHg concentrations in eggs. Instead, THg concentrations were measured and then it was assumed that THg was a reliable proxy for MeHg concentrations in eggs. The few prior studies that examined MeHg concentrations in bird eggs had relatively small sample sizes and did not span the entire range of observed Hg concentrations in wild bird eggs. We therefore conducted a detailed assessment of MeHg concentrations in eggs of two species over a 244-fold difference in Hg concentrations (0.03−7.33 μg/g fww).

Within American avocets and Forster's terns, MeHg concentrations were strongly correlated with THg concentrations in eggs (R^2 = 0.99), and the slope (m = 0.992) of the log−log regression indicated an almost perfect one-to-one relationship. Moreover, the percentage of THg in the MeHg form averaged 97% for American avocets and 96% for Forster's terns, and was not related to THg concentrations in individual eggs. The inclusion of species and its interaction with THg concentrations as factors did not improve either model's fit to the data, indicating that the relationship between MeHg and THg concentrations in eggs was robust among species, and shared a similar slope and intercept. Therefore, we confirmed that, both within and among species, THg concentrations were a very reliable proxy for MeHg concentrations in individual bird eggs over a wide range of environmentally relevant Hg exposure levels.

We then reviewed all the existing literature on MeHg concentrations in bird eggs and found that the percentage of THg in the MeHg form averaged 96% among 22 species of birds. Similar to our assessment within species, we found that the percentage of THg in the MeHg form was not related to THg concentrations in bird eggs among species. With this summarized data set, we documented that the percentage of THg in the MeHg form did not differ among foraging guilds and was not related to a species life history strategy. These outcomes were important in order for THg concentrations in eggs to be comparable among species. There are likely other ecological attributes that might alter the percentage of THg in the MeHg form in eggs. These include egg laying order, use of endogenous or exogenous reserves for egg formation, embryo age, habitat type, and site, but no patterns were apparent in the available data.

Although we found very strong correlations between MeHg and THg concentrations in eggs, the percentage of THg in the MeHg form in individual eggs ranged from 63% to 110% in American avocets and 76% to 116% in Forster's terns. Additionally, the mean percentage of THg in the MeHg form in eggs ranged from 82% to 111% among species. MeHg concentrations cannot actually exceed THg concentrations in the same egg, since THg includes both organic and inorganic forms of Hg. However, in practice, inherent variability in analytical results and subsampling of the egg homogenate during separate MeHg and THg determination procedures can cause the percentage of THg in the MeHg form to appear to exceed 100%. Because this sampling and analytical error has the same probability of being biased either high or low, we included estimates over 100% in our analyses rather than capping them at 100%. The variability in the percentage of THg in the MeHg form in eggs was relatively small, but tended to be greater at the lowest Hg exposure levels where small absolute differences between MeHg and THg concentrations could, nonetheless, result in large percent differences in the proportion of THg in the MeHg form.

Overall, our results strongly support the assumption that THg concentrations can be used as a reliable proxy for MeHg concentrations in bird eggs. Although there was some variation in the percentage of THg in the MeHg form in eggs, this variation was not related to Hg exposure levels or to major ecological or life history attributes among species and was more likely due to inherent variability in analytical results and subsampling of the egg. Therefore, THg concentrations in eggs can be used to estimate the risk of MeHg exposure to birds.

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Notes

The authors declare no competing financial interest.

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