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MERCURY DEMETHYLATION IN WATERBIRD LIVERS: DOSE–RESPONSE THRESHOLDS AND DIFFERENCES AMONG SPECIES

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Abstract—We assessed methylmercury (MeHg) demethylation in the livers of adults and chicks of four waterbird species that commonly breed in San Francisco Bay: American avocets, black-necked stilts, Caspian terns, and Forster's terns. In adults (all species combined), we found strong evidence for a threshold model where MeHg demethylation occurred above a hepatic total mercury concentration threshold of 8.51 ± 0.93 μg/g dry weight, and there was a strong decline in %MeHg values as total mercury (THg) concentrations increased above 8.51 μg/g dry weight. Conversely, there was no evidence for a demethylation threshold in chicks, and we found that %MeHg values declined linearly with increasing THg concentrations. For adults, we also found taxonomic differences in the demethylation responses, with avocets and stilts showing a higher demethylation rate than that of terns when concentrations exceeded the threshold, whereas terns had a lower demethylation threshold (7.48 ± 1.48 μg/g dry wt) than that of avocets and stilts (9.91 ± 1.29 μg/g dry wt). Finally, we assessed the role of selenium (Se) in the demethylation process. Selenium concentrations were positively correlated with inorganic Hg in livers of birds above the demethylation threshold but not below. This suggests that Se may act as a binding site for demethylated Hg and may reduce the potential for secondary toxicity. Our findings indicate that waterbirds demethylate mercury in their livers if exposure exceeds a threshold value and suggest that taxonomic differences in demethylation ability may be an important factor in evaluating species-specific risk to MeHg exposure. Further, we provide strong evidence for a threshold of approximately 8.5 μg/g dry weight of THg in the liver where demethylation is initiated.

Keywords—Mercury Demethlyation Liver Selenium Bird

INTRODUCTION

Mercury (Hg) contamination of wetlands and waterbodies throughout the world is increasingly causing concern because, in its methylated form, Hg is a highly potent neurotoxin [1] that readily bioaccumulates to elevated and potentially toxic levels in wildlife such as waterbirds [2–5]. Methylmercury (MeHg) exposure in wildlife occurs through diet and is rapidly distributed in the blood to target organs, including liver, brain, kidney, and muscle, where it accumulates over time [6] and may elicit a toxic response [3,4,7]. Assessing the toxic response of wildlife to MeHg in the field can be hampered by the complex pharmacodynamics of mercury [8] that often results in tissue-specific accumulation, depuration, sequestration, and detoxification [9]. Thus, it is important to consider the cycling and speciation of mercury in tissues such as liver to facilitate the interpretation of toxic risk.

The liver is the primary site of xenobiotic detoxification in wildlife. Consequently, several studies have suggested that MeHg may be detoxified in the liver of waterbirds [10–13]. These studies have shown that the proportion of Hg in liver comprised of MeHg (hereafter %MeHg) declines as the amount of total Hg (THg) in liver increases. These results indicate that MeHg detoxification in the liver occurs through a process of demethylation where MeHg is converted to inorganic Hg (IoHg) [10]. Recent evidence also suggests there may be a threshold value of THg concentrations in liver below which %MeHg values remain high and above which demethylation occurs and produces a larger proportion of IoHg [13]. However, the response of liver %MeHg in relation to liver THg levels remains unclear, and robust estimates of thresholds where MeHg begins to be demethylated are not known. Moreover, it is untested whether there are differences in demethylation thresholds between chicks and adult birds or among different species.

Selenium (Se) has long been thought to have a protective effect against MeHg toxicity [14,15] and has been proposed to play an important role in MeHg demethylation in avian livers, where both contaminants have been shown to coaccumulate [12,16]. However, the mechanism for the Hg–Se interaction is still not well understood. Previous research suggests that Se may act either as an initiator of demethylation [15] or as a receptor to bind IoHg after it has been demethylated, thus making it toxicologically inert [17].

In the present study, we examined liver demethylation in adults and chicks of four waterbird species that commonly breed in San Francisco Bay. Using an information theoretic approach, we evaluated the strength of several different model structures in describing the response of %MeHg values to increasing THg concentrations in liver. Specifically, for adults and chicks separately, we assessed whether %MeHg values were related to THg concentrations in a linear or nonlinear dose-dependent fashion. We compared several different model structures in which waterbirds might exhibit demethylation, including four changepoint models that depict a threshold. Our

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first model type was the null (Fig. 1a) and illustrates no MeHg demethylation in birds. In this model, the slope is zero and %MeHg values are unrelated to THg concentrations in liver. Our second model type was a straight-line model (linear regression, Fig. 1b) where the slope can be either positive or negative. Both straight-line responses could occur because MeHg may be demethylated with higher Hg exposure, or it may accumulate at a relatively higher rate than IoHg at higher exposures. Our third model type was a quadratic relationship (Fig. 1c) where %MeHg values increased smoothly with increasing THg values to a maximum, after which point the two variables were inversely related and the slope smoothly reversed with higher THg concentrations. Our fourth model type was a straight-line (or flat-line) changepoint model where %MeHg values increased or decreased with THg concentrations in a linear fashion (Fig. 1d) or were independent of THg levels (Fig. 1e) up to some threshold THg concentration, at which point the slope abruptly changed and %MeHg values decreased relatively more with each unit increase in THg. This model suggests that liver MeHg accumulates to a threshold concentration above which a demethylation response is initiated. Our fifth model type was a quadratic changepoint model (Figs. 1f and 1g), which is similar to previous changepoint models except that the post-threshold response followed a smoothly declining (quadratic) pattern rather than a sudden linear response. For threshold models, we also tested whether there were taxonomic differences in threshold concentrations or demethylation slopes. In summary, our objectives were to examine whether waterbirds can demethylate MeHg in the liver, describe the relationship between THg and MeHg in the liver, determine the threshold where demethylation begins, and assess the influence of Se on the demethylation process.

MATERIALS AND METHODS

Sample collection

In 2005 and 2006 we collected American avocet (*Recurvirostra americana*, hereafter avocet), black-necked stilt (*Himantopus mexicanus*, hereafter stilt), Caspian tern (*Hydroprogne caspia*, formerly *Sterna caspia*), and Forster's tern (*Sterna forsteri*) adults and avocet, stilt, and Forster's tern chicks at various locations throughout San Francisco Bay (37.8°N, 122.3°W), California, USA. We collected adults between February and July using shotguns with steel shot or high-powered air rifles as well as self-triggered treadle traps or remotely detonated bow nets (Northwoods, Rainer, WA, USA) and net launchers (Coda Enterprises, Mesa, AZ, USA). We collected chicks on or near their nesting colonies between July and August using hand nets during routine nest-monitoring activities or shotguns with steel shot. We excised livers using clean, acid-rinsed, stainless steel scalpels, scissors, and forceps, placed all tissue samples in polyethylene or glass containers, and stored them at −20°C until processing and analysis. Further details of necropsy methodology can be found in Eagles-Smith et al. [9]. All birds were captured and collected under the guidelines of U.S. Fish and Wildlife Service migratory bird and California Department of Fish and Game scientific collecting permits as well as the Animal Care and Use Committee, Western Ecological Research Center, U.S. Geological Survey.

Sample processing and chemical determination

We thawed livers at room temperature, rinsed them with deionized water to remove any surface contamination, and blotted them dry with lint-free wipes. We oven-dried the liver samples at 50 to 60°C for 48 h, or until they reached a constant mass, and weighed them before and after drying to determine their moisture content. Once dried, we homogenized the samples to a fine, uniform consistency using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) or by hand using a porcelain mortar and pestle.

We analyzed all samples for THg at the Davis Field Station Mercury Lab, U.S. Geological Survey, following the protocol described in Eagles-Smith et al. [9]. Quality assurance measures included analysis of two certified reference materials (either dogfish muscle tissue [DORM-2; National Research Council of Canada, Ottawa, ON, Canada], dogfish liver [DOLT-3; National Research Council of Canada], or lobster hepatopancreas [TORT-2; National Research Council of Canada, Ottawa, ON, Canada]), two system and method blanks, two duplicates, one matrix spike, and one matrix spike duplicate per batch. Recoveries averaged (± standard error [SE]) 103.63 ± 2.08% (n =116) and 98.84 ± 1.53% (n=116) for certified reference materials and calibration checks, respectively. Matrix spike recoveries averaged 95.1 ± 2.6%, N = 31. Absolute relative percent difference for duplicates averaged 2.6 ± 0.7%, and absolute relative percent difference for matrix spike duplicates averaged 3.7 ± 1.0%.

A subset of livers also was analyzed for MeHg at Battelle Marine Sciences Laboratory (Sequim, WA, USA) and Se at the Trace Elements Research Laboratory (Texas A&M University, College Station, TX, USA). Methylmercury values were determined using cold vapor atomic fluorescence following U.S. Environmental Protection Agency method 1630 (www.epa.gov/waterscience/methods/method/mercury/1630.pdf), and Se was determined using hydride generation, followed by atomic fluorescence. To provide an assessment of accuracy and precision, certified reference materials for MeHg and Se in tissues (DOLT-2 and National Institute of Standards and Technology 2976), matrix spikes, duplicate samples, and blanks were analyzed for quality control purposes. Recoveries for certified reference materials averaged 106.2 ± 1.83% (n = 57) and 106.31 ± 3.07% (n = 11) for MeHg and Se, respectively. Matrix spike recoveries were also within acceptable limits for both MeHg (98.80 ± 1.35%, n = 50) and Se (102.02 ± 7.04%, n = 11). Absolute relative percent difference for duplicates averaged 7.8 ± 1.5% (n = 42) for MeHg and 3.75 ± 1.22% (n = 11) for Se.

We report all tissue concentrations on a dry weight basis to control for differences in moisture content among individuals. However, to compare our results to wet-weight values reported in the literature we estimated the average moisture content (± SE) in livers for all species to be 65.82 ± 0.13%.

Statistical analysis

We conducted a tiered analysis separately by adults and chicks to assess mercury demethylation. Specifically, we first determined the best model explaining the relationship between %MeHg and THg concentrations for all species pooled and whether the best model indicated a threshold THg concentration at which %MeHg declined with increasing THg concentrations. Next, we evaluated whether the response in %MeHg to changes in THg differed among species. Third, we tested whether Se was related to changes in %MeHg.

We evaluated the response of %MeHg in liver to THg concentrations in liver using Akaike's information criterion (AIC) to select the most parsimonious model from an a priori set of

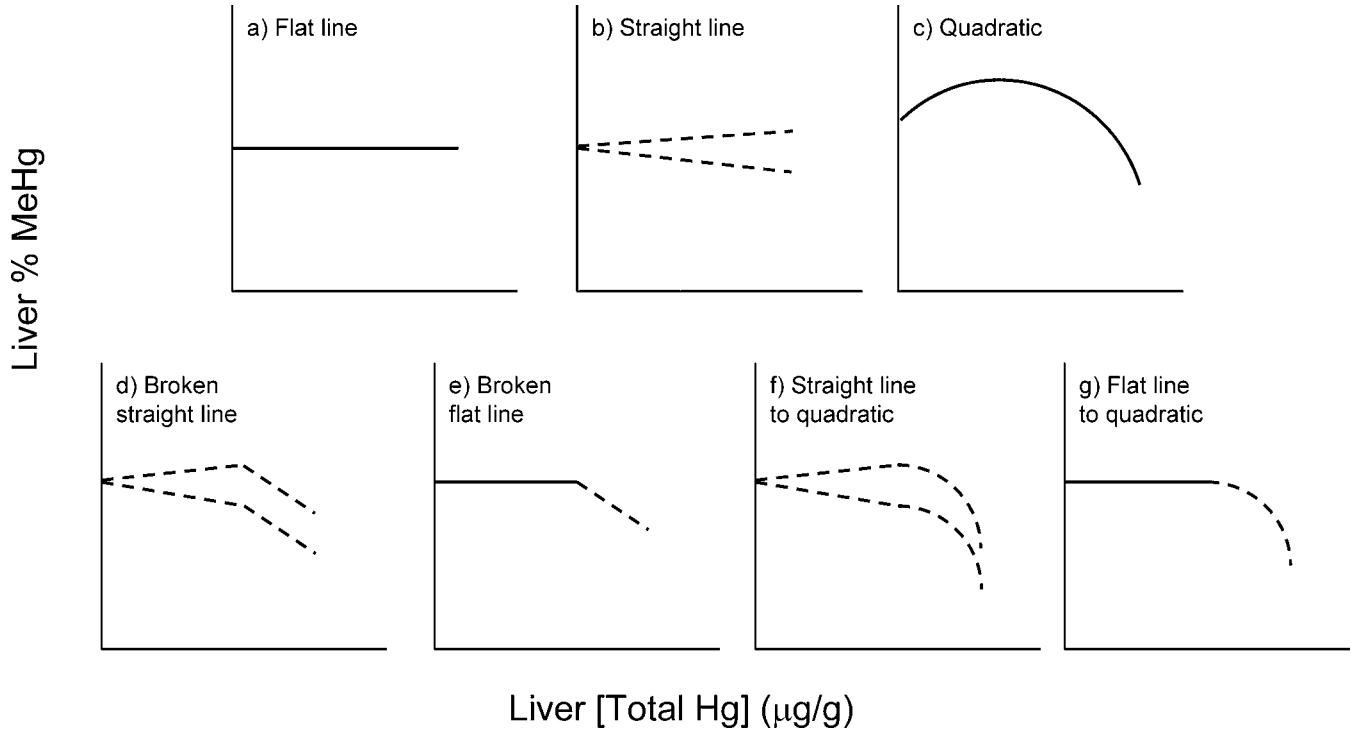

Fig. 1. Conceptual diagrams of modeled percent methylmercury (%MeHg) responses to total mercury (THg) concentrations compared for livers of American avocets, black-necked stilts, Caspian terns, and Forster's terns. Dashed lines indicate that a variable response is possible.

candidate models. We considered candidate models for biological importance when the difference between the second-order AIC (AIC*c*) of candidate models and the AIC*c* of the best model (ΔAIC_c) was ≤2.0. We calculated Akaike weights ($w_i = \exp[-\Delta AIC_c/2]/\Sigma_i \exp[-\Delta AIC_c/2]$) to assess the likelihood that the model with the lowest ΔAIC_c was actually the best model in the candidate set.

For the first-tier analysis, we used all species pooled to identify the overall model structure. We considered seven different a priori models using linear and nonlinear regression (PROC NLIN) with SAS version 9.1.2 [18]. The first response was a *flat-line* model (Fig. 1a) where the slope is zero, indicating that %MeHg is not related to changes in liver THg concentrations. The second response was a *straight-line* model (Fig. 1b) where the slope is any constant, corresponding to a pattern where the %MeHg in liver changes (either increases or decreases) at a linear rate as accumulation or exposure increases. The third response was a nonlinear *quadratic line* (Fig. 1c) where the slope changes smoothly with THg concentrations. The fourth response was a threshold *broken straight-line* model (Fig. 1d) where two lines are joined using changepoint regression (also called segmented regression [19]), resulting in a threshold where the slope of the relationship between %MeHg and THg changes. The fifth response type was a *broken flat-line* (Fig. 1e) model, which is similar to the preceding model, except that the initial slope is zero. The sixth and seventh response types were *straight-line change to quadratic* (Fig. 1f) and *flat-line change to quadratic* (Fig. 1g) threshold models, respectively. These two responses are similar to the preceding broken-line models, except that the threshold is followed by a quadratic function.

Using the selected model structure, we performed the second-tier analysis to assess differences among species by comparing various expansions of this model based on species-specific estimates of intercept, slope, and/or threshold (if applicable). Commonly, the default intercept in regression analysis is the value of y when $x = 0$. However, because we log-transformed our data prior to analysis, the default intercept is the %MeHg when THg concentration is 1 μg/g (log THg = 0). Therefore, we rescaled the x axis and defined the intercept as %MeHg where x is the minimum value in our dataset (0.32 μg/g).

For adult birds, the species-specific-estimates models failed to be properly estimated because we had inadequate data for at least one species. Therefore, we grouped species into two guilds (recurvirostrids: stilts and avocets; and terns: Forster's tern and Caspian tern) based on their phylogeny and foraging ecology, and we assessed differences in the response variable (%MeHg) between guilds. Because the most parsimonious, first-tier model for all adults (broken flat line) contained a changepoint (see *Results* section), we further evaluated the importance of guild by comparing six additional models. The first model structure was *no guild differences* where both guilds show the same demethylation response. We also evaluated the *complete differences* model where the intercept, threshold, and post-threshold slope all differed between guilds. Third, we examined a *parallel lines* model with different intercepts between guilds but the same slope. Finally, we assessed three variations of models in which both guilds were similar at low THg (same intercept) but diverged at higher THg concentrations. These variations included models where thresholds and post-threshold slopes differed, only thresholds differed, and only post-threshold slopes differed.

For chicks, the most parsimonious model from the first-tier analysis was linear and did not include a threshold (see *Results* section). We further considered species differences by comparing four different models that included either no species differences; *different intercepts, parallel slopes* where slopes are similar but intercepts differ among species; *common intercepts, uneven slopes* where intercepts are similar but slopes

Table 1. Ranking of candidate models describing response in liver percent methylmercury to changing liver total mercury concentrations in American avocet, black-necked stilt, Caspian tern, and Forster's tern adults and chicks from San Francisco Bay, California, USA[f]

Model no.	Model structure	*N*	*RSS*[a]	*k*[b]	Log-likelihood	AIC*c*[c]	ΔAIC*c*[d]	Akaike weight[e]
Adults—all models								
1	Flat line	309	59,179.9	2	−1,122.00	2,248.04	36.29	0.00
2	Straight line	309	50,751.4	3	−1,111.69	229.46	17.71	0.00
3	*Quadratic line*	*309*	*44,446.7*	*4*	*−1,102.79*	*2,213.81*	*1.96*	*0.17*
4	*Broken straight line*	*309*	*43,683.9*	*5*	*−1,101.63*	*2,213.45*	*1.71*	*0.19*
5	Straight line change to quadratic	309	44,305.5	5	−1,102.58	2,215.35	3.60	0.07
6	*Broken flat line*	*309*	*43,801.5*	*4*	*−1,101.51*	*2,211.45*	*0.00*	*0.44*
7	Flat line change to quadratic	309	44,852.6	4	−1,102.99	2,214.12	2.37	0.13
Chicks—all models								
1	Flat line	131	23,437.5	2	−424.91	853.91	3.06	0.09
2	*Straight line*	*131*	*21,405.8*	*3*	*−422.33*	*850.85*	*0.00*	*0.41*
3	*Quadratic line*	*131*	*21,074.3*	*4*	*−421.89*	*852.09*	*1.24*	*0.22*
4	Broken straight line	131	21,158.0	5	−422.0	854.48	3.63	0.07
5	Straight line change to quadratic	131	21,068.9	5	−421.88	584.24	3.39	0.07
6	Broken flat line	131	21,405.8	4	−422.33	852.98	2.13	0.14
7	Flat line change to quadratic**	131	–	4	–	–	–	–

[a] Residual sum of squares from the analysis of covariance model.
[b] The number of estimated parameters in the model including the variance.
[c] Akaike's Information Criterion.
[d] The difference in the value between AIC*c* of the current model and the value for the most parsimonious model.
[e] The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).
[f] Italicized rows indicate competing models with ΔAIC values suggestive of biological importance. Hypthens represent no data for those fields because the associated model failed to converge.
** Model failed to converge.

differ among species; and *complete differences* where both the slopes and intercepts differ among species.

Last, we examined the correlations between Se and residuals of the regression of %MeHg on THg to determine whether Se was associated with %MeHg when controlling for THg levels. We also examined correlations between Se and THg below and above the threshold, the ratio of Hg to Se in relation to the threshold, and the correlations between Se and IoHg separately for livers with THg below and above the threshold.

RESULTS

Demethylation threshold concentrations

We found that the most parsimonious models explaining the response of liver %MeHg to changing liver THg concentrations differed between life stages (Table 1). In adults, we found strong evidence for a threshold value at which the %MeHg in liver declined with increasing THg concentrations. The broken flat-line model was the best model, with an Akaike weight of 0.44 (Table 1). This model contains a changepoint estimated (±1 SE) at 8.20 ± 1.02 μg/g THg and suggests that for all species combined, a threshold existed at this concentration above which %MeHg declined with increasing liver THg concentrations (Fig. 2). There were two other competing models for adult birds (broken straight line and quadratic line), but the best model was 2.31 and 2.59 times as likely as each of these competing models, respectively (Table 1).

For chicks, the ''flat-line change to quadratic'' threshold model failed to converge due to the lack of this pattern in the data (Fig. 1f) and thus was excluded from further analyses. Of the remaining models, the straight-line model was 1.86 times as likely as the next-best model (quadratic line), and there was little evidence for the threshold models where the relationship between THg and %MeHg changed abruptly (Table 1). However, THg values in chick livers only ranged from approximately 0.3 to 15.3 μg/g dry weight, and most values were between 1 and 10 μg/g. Thus, our data may not have ranged enough to detect a threshold similar to that of adults whose THg concentrations ranged from 0.3 to 88.5 μg/g dry weight. Regardless, %MeHg values in chick livers of all species declined with increasing THg values at a rate of 5.51 ± 1.57% per log unit.

Species and guild differences

We tested whether the response of %MeHg to changes in liver THg concentrations differed among species or guilds. We

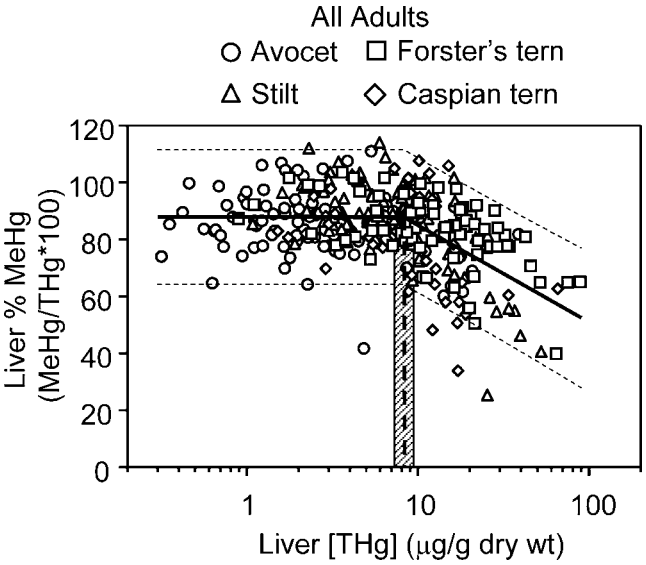

Fig. 2. Modeled mercury demethylation response (solid changepoint line) ± 95% confidence limits (dashed changepoint lines) in livers of adult American avocet, black-necked stilt, Caspian tern, and Forster's tern with all species combined. Vertical dashed line and shaded region indicate estimated mean (± standard error) demethylation threshold value. THg = total mercury.

Table 2. Ranking of candidate models comparing species differences in response of liver percent methylmercury to changing liver total mercury concentrations in American avocet, black-necked stilt, Caspian tern, and Forster's tern adults, and chicks from San Francisco Bay, California, USA[f]

Model no.	Model structure	*n*	*RSS*[a]	*k*[b]	Log-likelihood	AIC*c*[c]	ΔAIC*c*[d]	Akaike weight[e]
Adults								
1	No guild differences	309	43,801.5	4	−1,101.81	2,211.75	2.10	0.15
2	Different intercepts, thresholds, and slopes	309	42,232.4	7	−1,099.36	2,213.09	3.45	0.08
3	Parallel lines (intercepts differ)	309	43,740.8	5	−1,101.72	2,213.63	3.98	0.06
4	*Only thresholds and slopes differ*	*309*	*42,304.4*	*6*	*−1,099.48*	*2,211.23*	*1.58*	*0.19*
5	Only thresholds differ	309	43,439.1	5	−1,101.25	2,212.70	3.05	0.09
6	*Only slopes differ*	*309*	*42,461.3*	*5*	*−1,099.72*	*2,209.64*	*0.00*	*0.43*
Chicks								
1	*No species differences*	*131*	*21,405.8*	*3*	*−422.33*	*850.85*	*1.70*	*0.19*
2	*Parallel slopes (intercepts differ)*	*131*	*19,557.3*	*5*	*−419.76*	*850.00*	*0.86*	*0.30*
3	*Common intercepts (slopes differ)*	*131*	*19,264.8*	*5*	*−419.33*	*849.15*	*0.00*	*0.45*
4	Complete differences (intercepts and slopes differ)	131	19,195.6	7	−419.23	853.37	4.23	0.06

[a] Residual sum of squares from the analysis of covariance model.
[b] The number of estimated parameters in the model including the variance.
[c] Akaike's Information Criterion.
[d] The difference in the value between AIC*c* of the current model and the value for the most parsimonious model.
[e] The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).
[f] Italicized rows indicate competing models with ΔAIC values suggestive of biological importance.
** Model disqualified because it either failed to converge, or not all parameters could be estimated for at least one species.

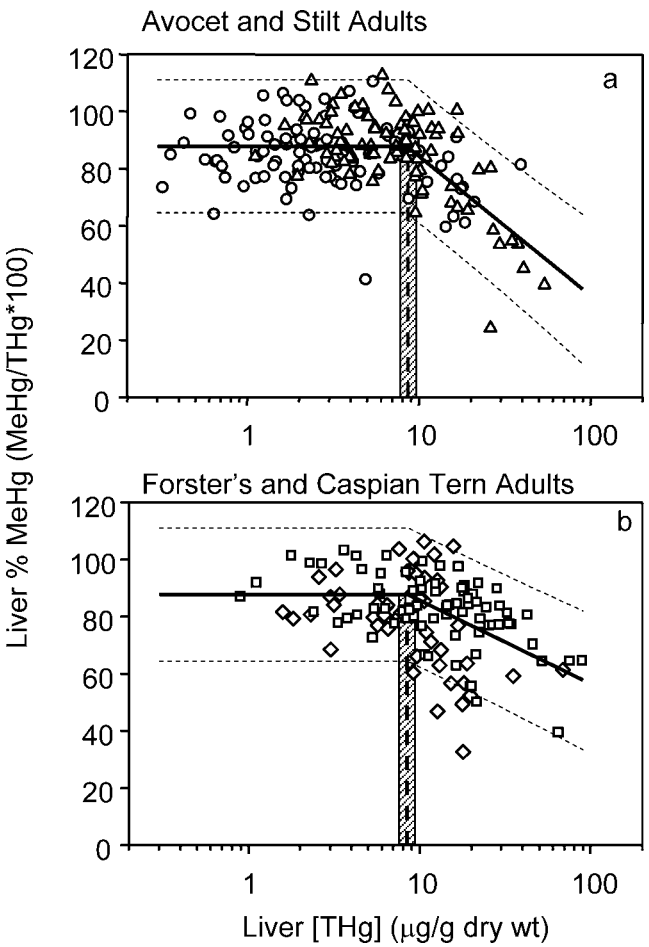

Fig. 3. Comparative demethylation responses in (**a**) American avocet and black-necked stilts and (**b**) Caspian and Forster's tern adults. Vertical dashed line and shaded region indicate estimated mean (± standard error) demethylation threshold value. Refer to Figure 2 for symbol key.

found strong evidence for differences between guilds in the rate of demethylation once the threshold was reached, as well as evidence for differences in the actual threshold values. The best two models (different slopes; different thresholds and slopes) had a combined Akaike weight of 0.62, and the "different slopes" model was 2.26 times as likely as the "different thresholds and slopes" model (Table 2). On the basis of the top model (different slopes), the %MeHg value was 87.76 ± 0.89% for both guilds when THg concentrations were low and began to decline when THg concentrations in the liver reached a common threshold concentration of 8.51 ± 0.93 μg/g. This threshold was slightly higher than that estimated with the first-order model (8.20 μg/g) because of the different structure associated with varying slopes between guilds. Subsequent to the threshold value, avocets and stilts had a steeper postbreakpoint slope (−21.18 ± 3.12, Fig. 3a) than that of terns (−12.71 ± 1.86, Fig. 3b). This indicated that nearly twice the proportion of MeHg was demethylated to IoHg in recurvirostrids than that in terns with each log unit increase in liver THg concentration.

Similar to the top model, the second-best model also had a common intercept with 87.72 ± 0.88% of the THg in the MeHg form at low THg concentrations and a steeper post-threshold slope for avocets and stilts (−24.94 ± 3.78) than that for terns (−11.35 ± 2.21). However, unlike the top model, the second-best model indicated that the threshold for terns occurred at a lower liver THg concentration (7.48 ± 1.48 μg/g) than that for avocets and stilts (9.91 ± 1.29 μg/g). These results suggest that active demethylation may have occurred with lower exposure levels in terns than that in avocets and stilts but that avocets and stilts were better able to demethylate Hg once the threshold was reached.

For chicks, there was little support for a threshold where the demethylation rate changed abruptly (Table 1). However, there was strong evidence for differences in the linear response of %MeHg to changing THg concentrations among species. The two top models (common intercept and parallel slopes) were close competitors and were 2.37 and 1.58 times as likely

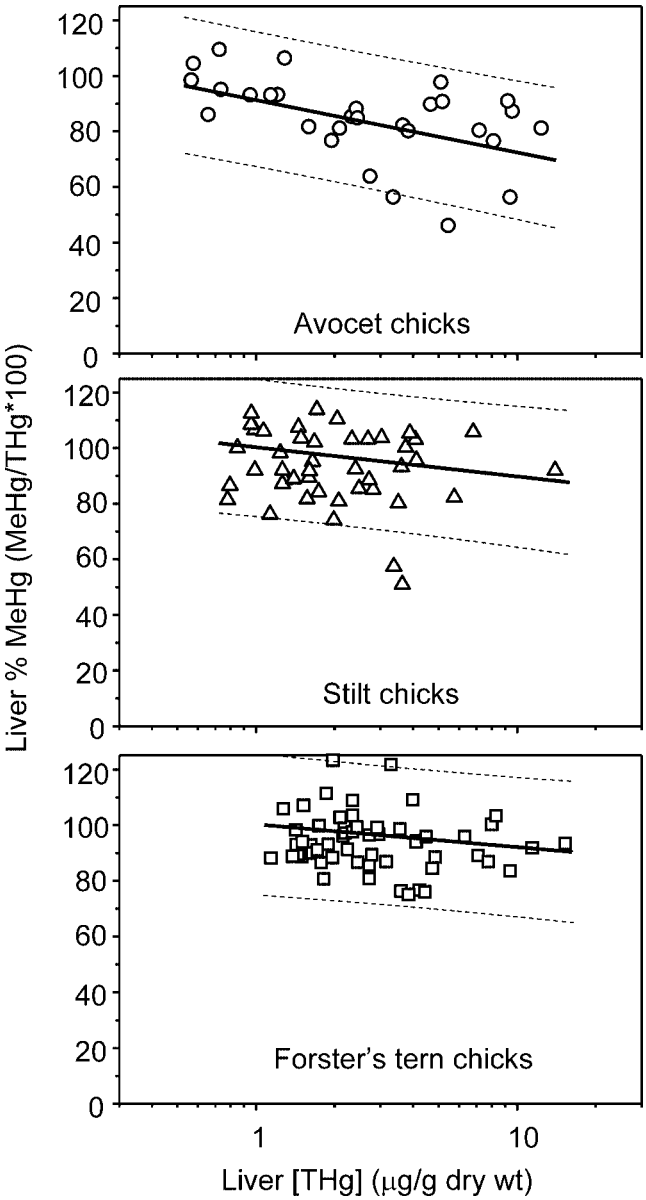

Fig. 4. Modeled mercury demethylation responses (solid line) ± 95% confidence limits (dashed lines) in livers of American avocet, black-necked stilt, and Forster's tern chicks. Refer to Figure 2 for symbol key. MeHg = methylmercury; THg = total mercury.

as the other competing model (no species differences, Table 2), respectively. The top model (common intercept) suggested that at the lowest liver THg concentrations (0.32 μg/g) liver THg was comprised of 101.88 ± 3.32% MeHg among all species. However, as liver THg concentrations increased, the %MeHg values changed at different rates among species. Avocets had the steepest decline with a decrease of 8.18 ± 1.67% MeHg per log unit of liver THg (Fig. 4), whereas stilts and Forster's tern %MeHg values declined by only 4.53 ± 1.89% and 3.56 ±1.59% per log unit, respectively (Fig. 4).

The common intercept model was 1.50 times as likely as the next-best model (parallel slopes), which indicated that there could be differences in %MeHg among species at the intercepts, but the relative differences in %MeHg among species remained consistent with increasing THg concentrations. According to this model, Forster's terns had the highest initial %MeHg value (106.00 ± 3.77%), followed by stilts (104.10 ± 3.37%), then avocets (96.37 ± 3.99%), and %MeHg declined commonly for all species by 5.57 ± 1.56% per log unit of liver THg. Finally, the third-best model suggested that there were no differences in slopes or intercepts among species, but there was substantially less support for this model. According to this model, %MeHg values started at 103.10 ± 3.39% and decreased by 5.51 ± 1.58% with each log unit increase in liver THg.

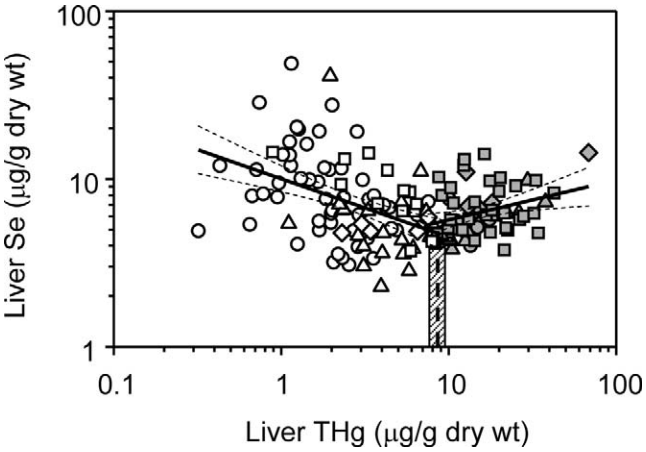

Fig. 5. Relationship between selenium (Se) and total mercury (THg) concentrations in livers of adult American avocets, black-necked stilts, Caspian terns, and Forster's terns. Open symbols represent birds with liver THg concentrations below the demethylation threshold; shaded symbols represent birds with liver THg concentrations exceeding the demethylation threshold. Vertical dashed line indicates estimated demethylation threshold derived from top changepoint model. Refer to Figure 2 for symbol key.

Selenium effects

We found no correlation between Se and the residuals from either of our top two threshold models for adults (linear regression; best model, $r^2 = 0.004$, $p = 0.42$, $n = 158$; second-best model, $r^2 = 0.003$, $p = 0.99$, $n = 158$), indicating that Se concentrations in the liver explained no additional variation in %MeHg after accounting for the variation associated with THg. We also found only a weak linear correlation between Se and THg concentrations in our liver samples with all species pooled (linear regression, $r^2 = 0.07$, $p = 0.001$, $n = 158$). However, when we assessed the relationship between Se and THg concentrations separately for data where THg was below and above the threshold (using the 8.51 μg/g threshold value derived when guild-specific values were allowed to differ, Table 2), we found strong relationships. Selenium concentrations were inversely related to THg levels at concentrations below our modeled demethylation threshold ($r^2 = 0.19$, $p < 0.0001$, $n = 96$), whereas they were positively correlated with THg at concentrations above the threshold ($r^2 = 0.12$, $p = 0.007$, $n = 62$, Fig. 5).

The molar ratio of Hg to Se ranged from 0.009 to 2.83 and increased with THg concentrations (Fig. 6). All liver samples with THg levels less than the demethylation threshold had Hg: Se molar ratios less than 1, indicating a molar excess of Se. As THg concentrations increased above the demethylation threshold, the ratio of most samples approached or exceeded 1, indicating molar equivalence between the two elements or even excess Hg. Using linear regression, we found that the slope was substantially lower (0.75 ± 0.09) for birds with THg

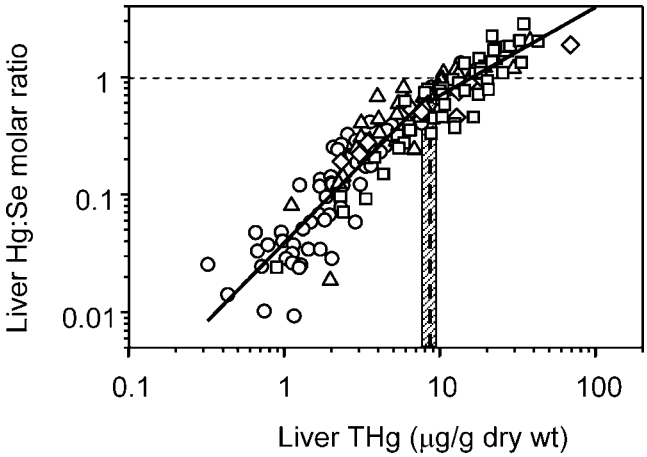

Fig. 6. Relationship between Hg:Se molar ratios and liver total mercury (THg) concentrations in adult American avocets, black-necked stilts, Caspian terns, and Forster's terns. Vertical dashed line and shaded region indicate estimated mean (± standard error) demethylation threshold value. Refer to Figure 2 for symbol key.

concentrations above the threshold than the slope for those with concentrations below the threshold (1.35 ± 0.07).

Consistent with these results, we also found that concentrations of IoHg in livers of birds with THg concentrations below the demethylation threshold were not correlated with levels of Se in the livers in either guild (avocets and stilts, $r^2 = 0.04$, $p = 0.05$, $n = 77$; Forster's and Caspian terns, $r^2 = 0.06$, $p = 0.31$, $n = 19$; Fig. 7). In contrast, IoHg concentrations were positively correlated with levels of Se in liver for both guilds when liver THg concentrations exceeded the threshold level (avocets and stilts, $r^2 = 0.54$, $p < 0.0001$, $n = 23$; Forster's and Caspian terns, $r^2 = 0.15$, $p = 0.01$, $n = 39$; Fig. 7).

DISCUSSION

Our results indicate a strong propensity for MeHg detoxification in adult avian livers through a process of demethylation, in which MeHg is converted to less toxic IoHg. In adults of all four species studied, we found that at low concentrations of THg in livers the proportion of Hg composed of MeHg was high (~88%) and remained constant, whereas there was a steep, linear decline in %MeHg with increasing THg concentrations in excess of approximately 8.20 μg/g dry weight. Interestingly, we found a different response in chicks, where %MeHg declined linearly with increasing THg concentrations in livers. We found no evidence for a threshold THg concentration where the rate of demethylation changed in chicks, although this lack of a threshold may have been due, at least in part, to a lack of higher Hg concentrations above the threshold that were more common in adults. For example, less than 7% of all chicks sampled had liver THg concentrations over 8.20 μg/g dry weight. Although we lacked sufficient data to model the adult biphasic response for each individual species, we did find evidence for a difference in demethylation response between the two guilds. Our best model indicated that at concentrations above the common threshold (8.51 μg/g when accounting for slope differences) avocets and stilts had a much steeper decline in %MeHg than terns. Moreover, a competing model suggested that the demethylation threshold in terns also may occur at a lower THg concentration (7.48 μg/g) than that in avocets and stilts (9.91 μg/g).

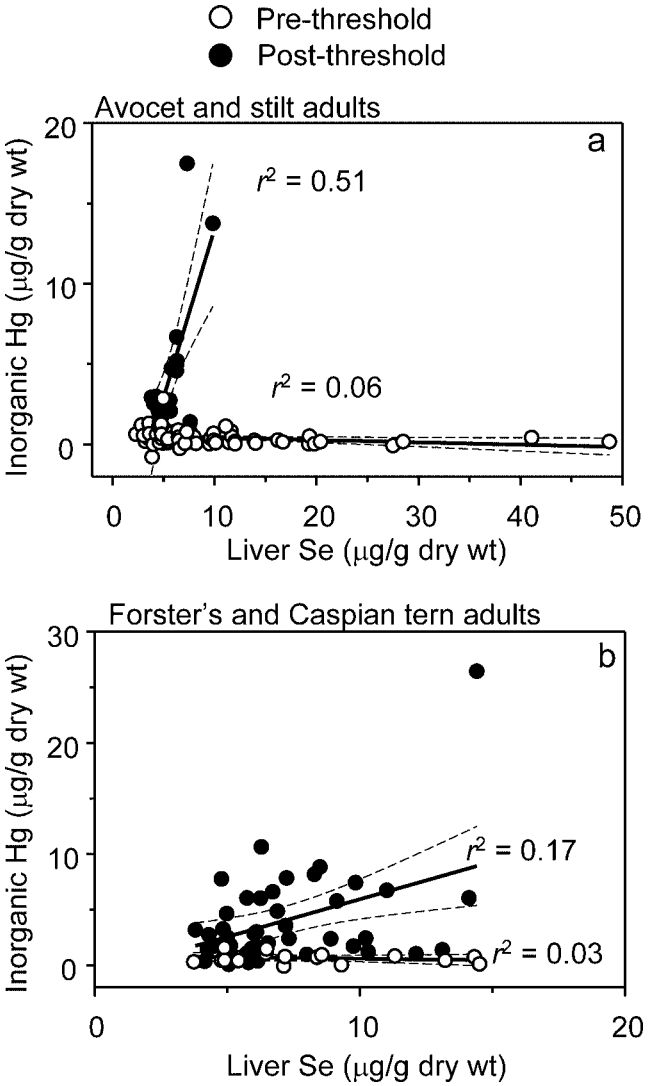

Fig. 7. Relationship between inorganic mercury and selenium of adult (**a**) American avocets and black-necked stilts and (**b**) Caspian and Forster's terns.

Selenium concentrations in the liver did not account for any additional variability in liver %MeHg values. Selenium concentrations also were not strongly correlated with THg concentrations across our entire range of THg values as has been reported in many other bird species [11–13,20,21]. Instead, we found a negative correlation between Se and THg below the demethylation threshold and a positive correlation above the threshold. Additionally, Hg:Se molar ratios were directly related to liver THg concentrations, and ratios approached one as THg concentrations approached our THg threshold for demethylation, indicating that demethylation may be initiated when molar equivalencies or excesses of Hg occur relative to Se. We also found that IoHg concentrations were poorly related with Se concentrations in birds with liver THg concentrations below the demethylation threshold, whereas birds with THg concentrations exceeding the demethylation threshold showed positive correlations between liver IoHg and liver Se concentrations. These results suggest that Se may be important for binding IoHg once it has been demethylated, thus making it unavailable for further tissue damage.

Our findings are consistent with studies on other seabirds [10] and piscivorous waterbirds [12,13], which have found that

at elevated THg concentrations the proportion of mercury in the liver that was comprised of MeHg (%MeHg) was relatively low despite the fact that much of their dietary mercury exposure was almost completely in the organic MeHg form. The ability to demethylate MeHg in the avian liver has long been proposed as a protective mechanism for reducing the toxicity of MeHg to the avian neurological system. However, few studies have assessed the variation of %MeHg values in liver over a wide range of liver THg concentrations. Most of those studies have also shown declines in liver %MeHg with increasing liver THg concentrations in birds. For example, Scheuhammer et al. [12] found that %MeHg concentrations in common loons (*Gavia immer*) and common mergansers (*Mergus merganser*) declined from 67 and 53%, respectively, in livers with THg concentrations between 0 and 5 μg/g dry weight to 45 and 38% between 6 and 10 μg/g, 34 and 28% between 11 and 20 μg/g, 25 and 21% between 21 and 30 μg/g, 8 and 17% between 31 and 40 μg/g, and 3 and 14% at concentrations greater than 40 μg/g. Similarly, several seabird species including the Atlantic petrel (*Pterodroma incerta*) [10], black-footed albatross (*Diomedea nigripes*), and Laysan albatross (*Diomedea immutabilis*) exhibited sharp declines in %MeHg values with increasing THg concentrations in their livers [11]. Alternatively, there was little relationship between %MeHg and THg in livers of adult double-crested cormorants (*Phalacrocorax auritus*), black-crowned night-herons (*Nycticorax nycticorax*), and snowy egrets (*Egretta thula*) when concentrations were <8 μg/g (wet wt), whereas %MeHg values were substantially lower and declined in a dose-dependent fashion as THg concentrations in the liver exceeded approximately 8 μg/g wet weight [13]. The apparent discrepancy between those studies is likely due to the fact that the seabird studies consisted of birds with exceptionally high THg concentrations (>50 μg/g dry wt) and likely did not capture the prethreshold levels, whereas the data from Henny et al. [13] cover a wide range of concentrations (1.12–222.16 μg/g wet wt). Our results also span a wide range of Hg concentrations (0.32–88.5 μg/g dry wt) and agree most closely with those of Henny et al. [13], suggesting that the energetic costs of initiating demethylation do not outweigh the costs of MeHg accumulation until concentrations become sufficiently elevated to cause toxicity.

Demethylation in chicks appeared to occur consistently across our range of THg concentrations, and unlike in adults we found little evidence for a THg threshold in chicks where the slope between %MeHg and THg changed abruptly. However, fewer than 7% of the THg concentrations in chick livers sampled exceeded the demethylation threshold level determined for adults, whereas THg concentrations in nearly half of the adult birds exceeded the demethylation threshold. Thus, we believe that Hg concentrations in the chicks that we sampled were likely too low to robustly assess the presence of a threshold. At low THg concentrations where we had overlap in levels between adults and chicks, the chick response in %MeHg did not agree with that in adults. We found a decline in %MeHg with increasing THg in chicks but not for adults. Similar findings were reported for fledgling wading birds in comparison to adults from Carson River, Nevada, USA [13]. Evidence of MeHg demethylation in livers of juvenile birds has also been reported for common loons [22], osprey (*Pandion haliaetus*) [23], and great egrets (*Ardea alba*) [24]. However, there are striking differences in the response among species. Percent MeHg values in loon chicks averaged 70% [22], and median values for osprey were 52% [23]. Despite this, there was little evidence of changes in %MeHg with increasing exposure. In MeHg-dosed great egret chicks, however, average %MeHg values in liver increased from 56% in birds fed control diets to 61 and 73% in the low (0.5 μg/g wet wt) and high (5 μg/g wet wt) dose groups, respectively [24]. These results suggest that chicks actively demethylate MeHg even at low exposure levels, and their ability to demethylate at high MeHg doses may be overwhelmed by excessive intake rates. Our results for chicks concur with these other studies showing that demethlyation occurs at low exposure levels and is consistent, without an abrupt change in demethylation rates, and that rates differ among species.

We found taxonomic differences in the demethylation response in adults. At THg concentrations above the demethylation threshold, we found that the slope of %MeHg versus THg for recurvirostrids (avocets and stilts) was steeper than that for terns (Forster's and Caspian terns). We also found evidence that demethylation was initiated at higher liver THg concentrations in avocets and stilts (9.91 μg/g dry wt) than that in terns (7.48 μg/g dry wt). These results suggest that MeHg demethylation may occur more rapidly in avocets and stilts than in terns but that avocets and stilts may not initiate demethylation until they reach relatively higher THg concentrations. Alternatively, avocets and stilts are likely exposed to MeHg at a lower rate than terns because they primarily forage at a lower trophic level on invertebrates [25,26], as opposed to fish, which are the main prey of terns [27,28]. Additionally, avocets and stilts are likely exposed to a relatively greater amount of dietary IoHg than terns due to their foraging ecology. For example, %MeHg levels in invertebrates such as those fed upon by avocets and stilts are highly variable [29], and a substantial proportion of invertebrate THg may be IoHg [30]. Conversely, nearly all of the Hg in fish is in the methyl form [31,32]; thus, terns and other fish-eating birds likely have very little dietary IoHg exposure. As a result, the MeHg pool in tern livers may be continually replenished at a relatively higher rate in comparison to that in avocets and stilts, which would likely result in a dampened response in declining %MeHg values even if demethylation was occurring at the same rate. This interpretation is also supported by the lower demethylation threshold level in terns than that in avocets and stilts, suggesting that terns may respond to their elevated exposure levels more readily than recurvirostrids, which likely accumulate MeHg more slowly.

Although little is known regarding the physiological mechanisms of MeHg demethylation in the avian liver [17,33], it is suspected to be an active, energy-dependent process. This hypothesis is consistent with the biphasic response in %MeHg relative to liver THg concentrations found in adults. At concentrations below toxicity thresholds, there is little benefit to expending energy in demethylation processes, whereas at Hg concentrations approaching or exceeding toxicity thresholds it becomes increasingly advantageous to direct energy to protective mechanisms. However, it is currently unclear whether differences in demethylation thresholds among species are due to differing species sensitivities to the toxic effects of MeHg or instead that species differ in their physiological ability to expend energy in the demethylation process. Recent studies have shown species differences in sensitivity to Hg through various endpoints such as embryo survival [34]; thus, it is unsurprising that we would find taxonomic differences in demethylation. Henny et al. [13] estimated that a dose-dependent relationship between %MeHg and THg in pooled livers of

double-crested cormorants, snowy egrets, and black-crowned night-herons was apparent at liver THg concentrations of about 7 to 8 μg/g wet weight (20–25 μg/g dry wt, assuming 66% moisture), and the slope of the relationship was −14.56. Similarly, Scheuhammer et al. [33] suggested that for wildlife in general IoHg species dominate hepatic Hg levels at concentrations above about 10 μg/g wet weight (30 μg/g dry wt). These values are substantially higher than those that we found (8.51 μg/g dry wt or 2.89 μg/g wet wt), indicating that those less sensitive to the toxicological effects of MeHg than the species studied here. Alternatively, the species studied here may be less able to utilize the protective abilities of Se at lower THg concentrations than other species in the literature.

Selenium has long been linked as an antagonist to the toxic effects of mercury in adult birds and other wildlife [15]. Selenium's role in MeHg detoxification has generally followed two hypotheses. One suggests that Se may enhance or enable detoxification processes by freeing methylmercury from its protein bonds, potentially making it available for demethylation [15]. The other hypothesis is that Se acts as a receptor for IoHg after it has been demethylated in the liver and binds with IoHg to form toxicologically inert Hg–Se compounds [17,35–38]. Our results generally support the latter hypothesis that Se does not enhance demethylation but instead provides a binding site after MeHg has been converted to IoHg for the formation of nontoxic, nonlabile Hg–Se compounds that are stored in the liver. Specifically, we found that little variation in our MeHg demethylation models was explained by Se. However, we found a significant, positive relationship between Se and IoHg concentrations in the livers of birds with high Hg concentrations, whereas there was no relationship between Se and IoHg concentrations in the livers of birds with low THg concentrations in liver. This suggests that once the threshold for demethylation is reached Se is important for receiving and potentially detoxifying the free IoHg. Henny et al. [13] found an even stronger positive correlation than our study between liver IoHg concentrations and Se in wading birds from Carson River, Nevada, USA. However, there was no comparison of pre- and post-threshold correlations.

Few other studies have assessed relationships between Se and IoHg, instead comparing Se with THg concentrations. In general, Se concentrations show strong linear correlations with THg [12,20,39]. These results are in sharp contrast to ours where we found that Se was negatively correlated with THg below the demethylation threshold and positively correlated with THg above the threshold. We are unaware of other examples where Se declines with increasing THg concentrations, and the mechanism for such a negative correlation is unclear. This trend may perhaps be due to routing of hepatic Se to other organs or merely the result of different foraging habitats among individuals, where some birds were feeding in high-Se, low-Hg sites. Recent work with common loons and bald eagles suggests that Se concentrations are poorly correlated with liver THg concentrations at THg values below 10 μg/g dry weight, whereas once THg concentrations exceed 10 μg/g dry weight they are strongly positively correlated [33]. Kim et al. [39] reported similar results for several seabird species. However, their results indicated that birds with THg concentrations <100 μg/g dry weight had a shallow slope and a poor correlation coefficient between THg and Se, whereas birds >100 μg/g THg dry weight had a steep slope and a high correlation coefficient. Together, these studies implicate Se as a very important component in the demethylation–sequestration process, particularly post-threshold. However, they also highlight that discrepancies among taxa still cannot be fully explained.

The results of our study are consistent with the hypothesis that MeHg toxicity can be reduced in livers of birds, that demethylation occurs across broad taxonomic groups, and that demethylation follows a dose–response threshold model. To our knowledge this is the first time that demethylation thresholds have been quantitatively modeled and compared to other potential functional response trajectories and compared among several species. Consequently, we showed that demethylation rates can differ among taxa and that there is evidence of taxonomic differences in the THg concentration threshold at which demethylation is initiated in the liver. Our study additionally lends further support to the role of Se as a Hg sequestration mechanism that works in concert with demethylation. However, our data suggest that, at least in some species of birds, the efficacy of selenium in this role may not always be as strong as that in other species and more research is needed to better understand these differences among taxa. Finally, our results suggest that assessments of avian risk to MeHg exposure should incorporate taxon-specific analysis of demethylation abilities.

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REFERENCES

1. Clarkson TW, Magos L. 2006. The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609–662.
2. Ackerman JT, Eagles-Smith CA, Takekawa JY, Bluso JD, Adelsbach TL. 2008. Mercury concentrations in blood and feathers of pre-breeding Forster's terns in relation to space use of San Francisco Bay, California, USA habitats. *Environ Toxicol Chem* 27: 897–908.
3. Burgess NM, Meyer MW. 2008. Methylmercury exposure associated with reduced productivity in common loons. *Ecotoxicology* 17:83–91.
4. Evers DC, Savoy LJ, DeSorbo CR, Yates DE, Hanson W, Taylor KM, Siegel LS, Cooley JH, Bank MS, Major A, Munney K, Mower BF, Vogel HS, Schoch N, Pokras M, Goodale MW, Fair J. 2007. Adverse effects from environmental mercury loads on breeding common loons. *Ecotoxicology* 17:69–81.
5. Scheuhammer AM, Basu N, Burgess NM, Elliot JT, Campbell GD, Wayland M, Champoux L, Rodrigue J. 2008. Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). *Ecotoxicology* 17:93–101.
6. Evers DC, Kaplan JD, Meyers MW, Reaman PS, Braselton WE, Major A, Burgess N, Scheuhammer AM. 1998. Geographic trend in mercury measured in common loon feathers and blood. *Environ Toxicol Chem* 17:173–183.
7. Hoffman DW, Ohlendorf HM, Marn CW, Pendleton GW. 1998. Association of mercury and selenium with altered glutathione metabolism and oxidative stress in diving ducks from the San Francisco Bay Region, USA. *Environ Toxicol Chem* 17:167–172.
8. Anderson DW, Suchanek TH, Eagles-Smith CA, Cahill T. 2008.

Mercury residues and productivity in ospreys and grebes in a mine-dominated ecosystem: Clear Lake, California. *Ecol Appl* 18:A227–A239.
9. Eagles-Smith CA, Ackerman JT, Adelsbach TL, Takekawa JY, Miles AK, Keister RA. 2008. Mercury correlations among six tissues for four waterbid species breeding in San Francisco Bay. *Environ Toxicol Chem* 27:2136–2153.
10. Thompson DR, Furness RW. 1989. The chemical form of mercury stored in south Atlantic seabirds. *Environ Pollut* 60:305–318.
11. Kim EY, Murakami T, Saeki K, Tatsukawa R. 1996. Mercury levels and its chemical form in tissues and organs of seabirds. *Arch Environ Contam Toxicol* 30:259–266.
12. Scheuhammer AM, Wong AHK, Bond D. 1998. Mercury and selenium accumulation in common loons (*Gavia immer*) and common mergansers (*Mergus merganser*) from eastern Canada. *Environ Toxicol Chem* 17:197–201.
13. Henny CJ, Hill EF, Hoffman DJ, Spalding MG, Grove RA. 2002. Nineteenth century mercury: Hazard to wading birds and cormorants of the Carson River, Nevada. *Ecotoxicology* 11:213–231.
14. Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P, Oh S, Hoekstra WG. 1972. Selenium: Relation to decreased toxicity of methylmercury added to diets containing tuna. *Science* 175: 1122–1124.
15. Sumino K, Yamamoto R, Kitamura S. 1977. A role of selenium against methylmercury toxicity. *Nature* 268:73–74.
16. Cuvin-Arelar MLA, Furness RW. 1991. Mercury and selenium interaction: A review. *Ecotoxicol Environ Saf* 21:348–364.
17. Ikemoto T, Kunito T, Tanaka H, Baba N, Miyazaki N, Tanabe S. 2004. Detoxification mechanism of heavy metals in marine mammals and seabirds: Interaction of selenium with mercury, silver, copper, zinc, and cadmium in liver. *Arch Environ Contam Toxicol* 47:402–413.
18. SAS Institute. 2005. SAS® version 9.1.2. Cary, NC, USA.
19. Draper NR, Smith H. 1998. *Applied Regression Analysis*, 3rd ed. John Wiley, New York, NY, USA.
20. Ohlendorf HM, Marois KC, Lowe RW, Harvey TE, Kelly PR. 1991. Trace elements and organochlorines in surf scoters from San Francisco Bay, 1985. *Environ Monit Assess* 18:105–122.
21. Bischoff K, Pichner J, Braselton WE, Counard C, Evers DC, Edwards WC. 2002. Mercury and selenium concentrations in livers and eggs of common loons (*Gavia immer*) from Minnesota. *Arch Environ Contam Toxicol* 42:71–76.
22. Kenow KP, Meyer MW, Hines RK, Karasov WH. 2007. Distribution and accumulation of mercury in tissues of captive-reared common loon (*Gavia immer*) chicks. *Environ Toxicol Chem* 26: 1047–1055.
23. Hopkins WA, Hopkins LB, Unrine JM, Snodgrass J, Elliot JD. 2007. Mercury concentrations in tissues of osprey from the Carolinas, USA. *J Wildl Manage* 71:1819–1829.
24. Spalding MG, Frederick PC, McGill HC, Bouton SN, McDowell LR. 2000. Methylmercury accumulation and its effects on growth and appetite in captive great egrets. *J Wildl Dis* 36:411–422.
25. Robinson JA, Oring LW, Skorupa JP, Boettcher R. 1997. American avocet (*Recurvirostra americana*). In Poole A, Gill F, eds, *The Birds of North America*, No 275. The Academy of Natural Sciences, Philadelphia, PA, USA, and The American Ornithologists' Union, Washington, DC, USA.
26. Robinson JA, Reed JM, Skorupa JP, Oring LW. 1999. Black-necked stilt (*Himantopus mexicanus*). In Poole A, Gill F, eds, *The Birds of North America*, No 449. The Academy of Natural Sciences, Philadelphia, PA, USA, and The American Ornithologists' Union, Washington, DC, USA.
27. Cuthbert FJ, Wires LR. 1999. Caspian tern (*Sterna caspia*). In Poole A, Gill F, eds, *The Birds of North America*, No 403. The Academy of Natural Sciences, Philadelphia, PA, USA, and The American Ornithologists' Union, Washington, DC, USA.
28. McNicholl MK, Lowther PE, Hall JA. 2001. Forster's tern (*Sterna forsteri*). In Poole A, Gill F, eds, *The Birds of North America*, No 595. The Academy of Natural Sciences, Philadelphia, PA, USA, and The American Ornithologists' Union, Washington, DC, USA.
29. Muhaya BBM, Leermakers M, Baeyens W. 1997. Total mercury and methylmercury in sediments and in the polychaete *Nereis diversicolor* at Groot Buitenschoor (Scheldt Estuary, Belgium). *Water Air Soil Pollut* 94:109–123.
30. Suchanek TH, Eagles-Smith CA, Slotton DG, Harner EJ, Adam DP, Colwell AE, Anderson NL, Woodward DL. 2008. Mine-derived mercury: Effects on lower trophic species in Clear Lake, California. *Ecol Appl*. 18:A158–A176.
31. Bloom NS. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can J Fish Aquat Sci* 49:1010–1017.
32. Gonul LT, Kucuksezgin F. 2007. Mercury accumulation and speciation in the muscle of red mullet (*Mullus barbatus*) and annular sea bream (*Diplodus annularis*) from Izmir Bay (Eastern Aegean). *Mar Pollut Bull* 54:1962–1968.
33. Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–18.
34. Heinz GH, Hoffman DJ, Klimstra JD, Stebbins KR, Kondrad SL, Erwin CA. 2009. Species differences in the sensitivity of avian embryos to methylmercury. *Arch Environ Contam Toxicol* 56: 129–138.
35. Cabanero AI, Madrid Y, Camara C. 2005. Study of mercury-selenium interaction in chicken liver by size exclusion chromatography inductively coupled plasma mass sprectrometry. *J Anal At Spectrom* 20:847–855.
36. Cabanero AI, Madrid A, Camara C. 2006. Selenium long-term administration and its effect on mercury toxicity. *J Agric Food Chem* 54:4461–4468.
37. Caurant F, Navarro M, Amiard J. 1996. Mercury in pilot whales: Possible limits to the detoxification process. *Sci Total Environ* 186:95–104.
38. Palmisano F, Cardellicchio N, Zambonin PG. 1995. Speciation of mercury in dolphin liver: A two-stage mechanism for the demethylation accumulation process and role of selenium. *Mar Environ Res* 40:109–121.
39. Kim EY, Saeki K, Tanabe S, Tanaka H, Tatsukawa R. 1996. Specific accumulation of mercury and selenium in seabirds. *Environ Pollut* 94:261–265.